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TUMOR IMMUNOLOGY: FUNCTION AND CHARACTERIZATION OF IMMUNE CELLS AND ANTIBODIES

JUNE 1981

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CURRENT CANCER RESEARCH

I. LYMPHOID CELLS

A. T LYMPHOCYTES

1. T CELL DIFFERENTIATION AND MATURATION

1. T CELL GROWTH AND DIFFERENTIATION IN LEUKEMIA

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We are undertaking an analysis of the potential for T cell differentiation and growth of precursor cells mainly in patients with acute lymphocytic leukemia. These cells have been shown to be incapable of differentiation in vivo. For this study, CM will be used to induce differentiation and development of the precursor cells associated with the null lymphocytes. Comparing the normal and patient T cell developmental sequence as delineated by emergence of cellular markers, subclasses and functions, we hope to understand the T cell developmental process and the nature of the abnormalities in leukemia. Employing the differentiation and proliferation assays, we will be able to analyze the stem cells, serum, growth and differentiation factors and their interactions in relation to the differentiation block and other abnormalities of the disease. We hope that this will facilitate a better definition of the leukemias and improve understanding of the regulation of hematopoiesis and the immune system in fundamental terms. We also aim at better detection and understanding of the factors involved in differentiation at the molecular level and specificities of its activity. This basic information will ultimately contribute to better treatment and perhaps prevention of the leukemias and other malignancies.

2. MATURATION OF T-LYMPHOCYTES IN BONE MARROW FROM NORMALS AND FROM PATIENTS WITH DIFFERENT TYPES OF ACUTE LYMPHOBLASTIC LEUKEMIA

de Vaan, G. A., Bakkeren, J. A., Schretlen, E. D., Katholieke Universiteit Nijmegen, Dept. of Pediatrics, *Geert Grooteplein Zuid 20, Nijmegen, Netherlands*

OBJECTIVE: Unbridled proliferation and the lack of normal cell maturation are essential characteristics of acute leukemia. The different subtypes of acute lymphoblastic leukemia (ALL) may be correlated with the various stages of maturation of the lymphoid pattern in normal bone marrow. Fractionation of bone marrow via gradient density centrifugation of normal and leukemic bone marrow may provide information for a comparative study.

APPROACH: Various methods of density gradient centrifugation are investigated to fractionate normal and leukemic bone marrow. A discontinuous bovine serum albumin (BSA) gradient and a continuous Percoll gradient are studied. For identification of the different subfractions, besides the immunological characteristics and antigenic determinants on the cell surface, the proliferative capacity of the cells is also determined. Leukemic bone marrow cells from children with various lympho- and myelo-proliferative diseases are included in the study.

PROGRESS: By centrifugation over a discontinuous BSA density gradient good reproducibility of the partition of the bone marrow cell population was obtained with a control group of 12 children with ALL in complete remission and after cessation of all antileukemic therapy. In 9 children with various lympho- or myelo-proliferative malignancies, similar fractionation revealed a different cell partition pattern as compared with the control group. Similar experiments using a continuous Percoll gradient are in progress.

BIBLIOGRAPHIC REFERENCES: Bakkeren, J.A.J.M., de Vaan, G.A.M., Utility of density gradient centrifugation of leukemic bone marrow. Med. Ped. Oncol., 8,83-96 (1980).

3. PROLIFERATION AND DIFFERENTIATION OF HUMAN T LYMPHOCYTES IN VITRO UNDER THE INFLUENCE OF STIMULATORY PROTEINS

Wu, A. M., University of Toronto, Dept. of Anatomy, *Medical Sciences Bldg., Toronto, Ontario*, M5S 1A8, Canada

OBJECTIVES: Specific interactions between regulatory factors and their target cells are the basic mechanisms underlying regulation of normal hemopoietic cell differentiation. Leukemia-lymphoma and other hematological illnesses could be the results of disturbance of these specific cell-factor interactions.

APPROACH: To elucidate the mechanism of the cell-factor interaction, we used the differentiation of human T progenitor cells as the model system. T lymphocytes and their progenitor cells were grown long-term in a liquid culture in the presence of regulatory activity termed T lymphocyte growth stimulatory activity (TL-GSA) or grown clonally as T cell colonies in semi-solid medium. In the suspension culture, 6 kinds of functional progenies were detected. These included cytotoxic T cells, helper and suppressor T cells for B cell differentiation, responder for mixed leukocyte reaction, T cell colony forming unit (TL-CFU) and granulocyte macrophage colony stimulatory activity (GM-CSA) producing cells.

PROGRESS: Some of our findings are: 1) There is a common progenitor cell for human and B and T lymphocytes. 2) The factor-producing cells and factor-responding cells belong to the same clone. 3) The helper T progenitor cells in the suspension culture can generate at least two types of antigen-specific helper T cells (the antigens used were sheep erythrocyte and ovalbumin). 4) The cytotoxic T cells in the suspension and in the T cell colonies are capable of lysing at least three different cultured tumor cell lines. Since these cells are E positive and Fc negative cells, they are different from conventional natural killers. 5) There are two types of TL-CFU - one is the more mature TL-CFU which requires only PHA for colony formation; the less mature one requires an additional activity termed colony-promoting activity to convert them to sensitivity to PHA. Both types of TL-CFU can renew themselves extensively in culture according to a stochastic process and this process is regulated by an activity termed self-renewal enhancing activity (SREA). SREAs are nondialyzable glycoproteins with a mannoside terminal.

4. LYMPHOCYTE FORMATION AND DIFFERENTIATION IN VITRO

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This project involves the study of the formation and differentiation of lymphoid precursor cells from murine and human bone marrow cells cultured in liquid and in semisolid media. Attempts to expand lymphoid cell lines will include stimulation of cultures with colony stimulating factor (CSF), T cell growth factor (IL-2) and supernates from mitogen activated lymphoid cell cultures. Cells proliferating in vitro will be assayed for clonability, T and B lymphocyte activity (including helper/suppressor activity and cytotoxic activity) and immunoglobulin expression and production. The phenotypical characteristics of proliferating cells or cell lines will be studied by means of immunofluorescence techniques including monoclonal antibody labeling and FACS analysis.

5. CELL DIFFERENTIATION STUDIES IN CANCER IMMUNOBIOLOGY

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The first purpose of these investigations is to define some of the cellular and molecular aspects of T- and B-cell differentiation through comparative studies in mice, rabbits, chickens, frogs and humans. Integrated studies of individuals with leukemias, lymphomas and immunodeficiencies will be the other major focus. Using immunofluorescent and biochemical techniques, we will examine the nature of the immunoglobulin components expressed by pre-B cells from normal tissues, cell lines and pre-B hybridomas, with emphasis on the genetic diversity of variable regions of IgM heavy chains. Anti-idiotype antibodies and isoelectric focusing will be used to examine normal generation of clonal diversity, the cellular basis of pauci-clonal antibody responses and the extent of clonal involvement of murine plasmacytomas and a spectrum of B-cell malignancies in humans. Adoptive transfer and/or in vitro model systems will also be used in this examination. Generation of isotype diversity will be examined in frogs (IgM and IgG), chickens (IgD) and man (IgA1 and IgA2). The immunosuppressive effects of anti-Ia will be investigated along with phylogenetic aspects of Ia structure and function. We will continue to examine human T-cell subpopulations, especially TM and TG cells, and search for VH expression by human T cells. Antibodies to clathrin will be used to study the role of this highly conserved protein in lymphocyte activation and differentiation. Hybridoma antibodies will play a central role in these studies, and somatic cell hybridization used to examine generation of VH and isotype diversity and to map genes for human immunoglobulins.

6. IMMUNOBIOLOGY OF EARLY LYMPHOCYTE DEVELOPMENT

Sato, V. L., Harvard University, Cambridge Campus, School of Arts & Sciences, Dept. of Biology, *Cambridge Station, Cambridge, Massachusetts*, 02138, United States of America

A significant portion of lymphocyte differentiation occurs in the bone marrow where lymphocytes first separate themselves from other hemopoietic cells and where T cells initially segregate themselves from B cells. Lymphocytes therefore make important maturational decisions in this compartment of differentiation. The heterogeneity of the bone marrow cell population has made dissection of this compartment difficult, and our approach has been to define cell surface antigens to identify different cell types and to find tumor analogs that could provide useful homogeneous clones of cells. We have data demonstrating that Abelson murine leukemia virus can be used profitably in two ways: 1. To mark hemopoietic and lymphoid progenitor cells; 2. to provide cloned transformed lines of such progenitors.

We propose to extend these studies with new attention to the in vitro induction of the cell lines to express more differentiated surface phenotypes.

7. IN VITRO THYMUS-DEPENDENT LYMPHOCYTE DIFFERENTIATION

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T lymphocytes are a major arm of the immune system. Immature lymphoid precursor cells are matured in the thymus by contact with non-lymphoid influencing cells to yield T cells with mature functional capabilities, including the ability to distinguish self from non-self. There is evidence to indicate that this differentiation takes place in steps mediated by different kinds of influencing cells located in different parts of the thymus. In order to study the nature, number and order of these steps, an in vitro differentiation system will be established using cultured thymus influencing cells and purified precursor cells at various stages of differentiation. Once the order of the steps is apparent, subpopulations of influencing cells will be separated and used to study single steps in detail, with the eventual goal of understanding the mechanism of the commitment to self. The MRC Unit at Oxford has a highly developed rat system with the appropriate genetics, immunological assays, monoclonal antibodies and cell separation technology available.

8. ROLE OF THE THYMUS IN GENERATION OF THE SELF-MHC SPECIFIC T CELL REPERTOIRE

Kruisbeek, A., Singer, A., Hodes, R. J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Immunology Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

In order to ascertain that the thymus is the organ in which the T cell self-MHC specific repertoire is determined, the MHC specificity of cytotoxic precursor cells differentiating in the thymuses of radiation bone marrow chimeras was assessed. The results of the experiments demonstrated that thymocytes from A-yields-AxB chimeras were capable of recognizing TNP in association with both A and B MHC determinants, and that thymocytes from AxB-yields-A chimeras recognized TNP only in association with A, not B, MHC determinants. Thus, the earliest donor T cell in chimeric mice already displayed self-MHC recognition restricted to the host thymic MHC determinants. These results are consistent with the thymus being the site where MHC restrictions of T cell recognition are determined, and virtually excluded the possibility that MHC restriction is only a post-thymic event.

9. THE CONTROL OF LYMPHOCYTE PROLIFERATION AND DIFFERENTIATION

Paetkau, V. H., University of Alberta, Dept. of Biochemistry, *Edmonton, Alberta*, T6G 2E1, Canada

OBJECTIVES: The objectives are to understand the mechanisms whereby leukocyte populations control their own proliferation and differentiation. The biochemical nature of intercellular signals, and their mode of action on lymphocytes, are of primary interest. Model systems for inducing cell-mediated toxicity toward cancer cells are to be developed. Understanding the controls operating to regulate the extent of proliferation, and the development of specialized responses like the immune reaction, may lead to a useful approach to controlling lymphocyte functions.

APPROACH: The present focus is on the characterization and mode of action of a particular lymphokine, which we have called co-stimulator, and which appears to be a second signal component of stimulating a number of lymphocyte responses. The effects of controlling the level of co-stimulator in model systems such as CTL and AFC generation are being studied. The relationship of this lymphokine to others described in the literature is to be investigated.

PROGRESS: Co-stimulator is a protein of MW 30,500, which is essential in generating mitogenic responses in murine thymocytes, in producing CTL to alloantigens or tumor antigens in vitro, and in obtaining AFCs from T cell-deprived populations of spleen cells. It is a product of T helper cells activated by 2 signals: a mitogen (or antigen) and a soluble macrophage product, lymphocte activating factor. Co-stimulator, in the presence of tumor antigen, stimulates a strong cytotoxic antitumor response when added to cultures of spleen cells from mice bearing normally fatal syngeneic tumors. These activated cytotoxic cells, injected into identical tumor-bearing mice, promote tumor clearance and enhance survival.

10. REGULATION OF T-CELL PROLIFERATION AND DIFFERENTIATION

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T-cell growth factor (TCGF) is a mitogenic protein released from mononuclear cells upon lectin or antigen stimulation. Detailed studies have shown that TCGF, which allows for the continuous proliferative culture of T-cells, acts as the mitogenic stimulus after lectin/antigen activation. The aims of the current project are to purify TCGF, develop a radiolabeled TCGF-cell binding assay, and produce monoclonal antibodies to TCGF and to the TCGF receptor. Other experimentation will be directed toward exploring the in vivo significance of TCGF and defining the mechanisms of TCGF production and action. It is hoped that these studies will provide new information and technical approaches which will lead to an increased understanding of the regulation of T-cells in both physiologic and pathophysiologic states.

BIBLIOGRAPHIC REFERENCES: P.E. Baker, S. Gillis, and K.A. Smith. Monoclonal cytolytic T-cell lines. J. Exp. Med. 149:273, 1979. S. Gillis, N.A. Union, P.E. Baker, and K.A. Smith. The in vitro generation and sustained culture of nude mouse cytolytic T-lymphocytes. J. Exp. Med. 149:1460, 1979.

11. ROLE OF THYMOSIN PEPTIDES IN T-CELL DIFFERENTIATION

Goldstein, A. L., George Washington University, School of Medicine & Health Sciences, Dept. of Biochemistry, *2121 Eye St. N.W., Washington, District of Columbia*, 20037, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

12. T LYMPHOCYTE DIFFERENTIATION AND LEUKEMOGENESIS

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This is part of a broader project. A summary of this subproject is not available.

13. STUDIES ON ONTOGENY AND SPECIFICITY OF VIRUS-SPECIFIC CYTOTOXIC T CELLS

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OBJECTIVE: To analyze T cell specificity and nature of T cell receptors during ontogeny and at the level of effector T cells; to study immune response gene regulation of T cells and to evaluate their influence in immunologically mediated disease.

APPROACH: Irradiation - Bone marrow chimeras or thymus chimeras are used to study T cell maturation in vivo; in particular the respective roles of thymic epithelial cells and of antigen-presenting cells in the thymus are examined. T cell lines and T cell clones with defined cytotoxic effector specificity against various viruses are being established to provide the basis for biochemical and structural analysis to evaluate whether T cells have one or two receptors. Such T cells will be studied in vivo with respect to immuno-protective as well as immunopathological effector functions.

14. CLONAL GROWTH AND MATURATION OF NORMAL AND MALIGNANT LYMPHOID CELLS

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Growth factors contained in concanavalin A (Con A)-conditioned spleen cell culture media maintain exponential growth of T cell blasts derived from stimulation with mitogenic lectins or antigens. Such growth factors are T cell blast-specific and independent of the mitogenic lectin used for its production. T cell blasts, but not small resting T cells, display receptors on the outer cell membrane for such growth factors. T cell blasts responding to such growth factors do not themselves produce these factors, nor do they respond to mitogenic lectins or antigens even in the presence of syngeneic, Ia-bearing adherent cells. We have shown that such cells constitute a mixture of conventional killer T cells and natural killer cells able to kill certain sensitive tumor targets as well as teratocarcinoma cell lines. The growth-promoting activity for these two types of cytolytic cells is independent of interferon.

15. NORMAL AND NEOPLASTIC T AND B LYMPHOCYTE MATURATION

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This project is a continuation of our long-term study on the cellular and molecular mechanisms of normal and neoplastic T and B lymphocyte maturation. Our first goal is to study differentiation of T lymphocytes, with a major focus on analyzing the number and type of lineages beginning in the bone marrow and ending in peripheral immunocompetent T cell pools. Another aspect of normal T cell maturation is the analysis of how thymic epithelial cells create a microenvironment for the selection and/or maturation of immunocompetent T cell subsets. Immunochemical and molecular biological experiments are planned to elucidate the characteristics of T cell receptors and the genes coding for these receptors. A novel hypothesis tested in this grant is that neoplastic transformation of T lymphocytes by murine leukemia viruses is limited to that subset of T cells having antigen-specific receptors for envelope glycoproteins of these viruses. We shall test this notion by using various immunochemical reagents to define virus receptors and antigen-specific T cell receptors for these viral envelope glycoproteins. In a third line of work we shall examine another class of T cell receptors involved in the homing and organization of lymphoid tissues in normal hosts, and the metastasis of lymphomas in tumor bearing hosts. We are referring to lymphocyte receptors for particular blood vessels which govern the homing of lymphocytes to various parts of the body, and our analysis includes a biological and a biochemical characterization of these receptors. Finally, we shall look closely at normal and neoplastic B cell maturation, with special emphasis on studying isotypes as markers of B cell maturational lineages, and the organization of heavy chain constant and variable region genes during this maturation in both normal and neoplastic B cells.

16. THYMUS CELL MATURATION AND LEUKEMOGENESIS IN MICE

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In the present proposal an indirect effect of oncogenic agents in leukemogenesis is hypothesized. It is possible that carcinogens cause alterations in the proper maturation and differentiation of T cells, resulting in an imbalance in the immune system which then leads to malignancy. It is assumed that any defect is subtle. Therefore, it is proposed to use more refined immunological techniques to study the normal maturation and differentiation of T cells. Allogeneic effect factor (AEF), which has been shown to have a profound effect on the differentiation of B cells, will be used as a probe for study of differentiation of pluripotential bone marrow stem cells into the lymphoid line. Basic understanding of stem cell commitment to T cell development will allow study of anomalies leading to malignancy.

17. DEVELOPMENT OF THYMIC LYMPHOCYTES

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The proposed investigation is a study of the development of thymic lymphocytes from hematopoietic stem cells. A population of cells enriched in prothymocytes will be prepared by isopycnic centrifugation, differential killing and differential adhesiveness. These cells will be assayed by their ability to repopulate the thymus of irradiated mice and by their response to the purified thymic hormone thymopoietin. The state of differentiation of the prothymocyte and the degree of maturation induced by thymopoietin will be evaluated and the relationship of these cells to the progenitors of B lymphocytes determined.

BIBLIOGRAPHIC REFERENCES: Basch, R.S., Kadish, J.L., and Goldstein, G. Hematopoietic thymocyte precursors. IV. Enrichment of the precursors and evidence for heterogeneity. J. Exp. Med. 147:1843, 1978. Jeng, M.W., Finegold, M., Basch, R.S., and Lamm, M.E. Immunoelectron microscopic studies of thymus-leukaemia (TL) antigens on mitochondrial membranes obtained from lymphoid cells. Lab. Invest. 38:41, 1978.

18. IMMUNITY IN T CELL DEFICIENT MICE

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The overall objective of this proposal is to study the function of thymus derived lymphocytes (T cells) in mice in which T cells mature in the absence of a functioning thymus. Two essential questions are being explored. One is whether T cell functions can be induced in thymus deficient nude mice. The other is whether T cell function is normal and specific. Both questions are important from both a practical and a theoretical point of view. Thus, it would be useful if T cell function could be restored in thymus deficient animals by certain factors, and it would be of considerable importance to know whether the thymus has a mere humoral or an additional selective function.

Recent experiments have shown that conditioned media (CM) prepared from spleen cells stimulated with T cell mitogens may induce the differentiation and proliferation of functional T cells in nude spleen cells. Furthermore, certain effector cells - for instance, those with natural killer activity - can be grown from nude spleen cells using CM. The specificity and function of T cells and of permanent lines derived from nude spleen will be assessed. Furthermore, the ability of these cells or cell lines to restore immunoincompetent hosts will be examined.

19. T CELL DEVELOPMENT - IMMUNOGENETICS, DEFECTS, THERAPY

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Proportions ('profiles') of the three main mouse T cell sets, Ly123:Ly1:Ly23, are characteristically distored in immune-deviant hairless (hr) mutant mice (increased Ly123: decreased Ly1), and after thymectomy (decreased Ly123: increased Ly1).

We propose to define these models further, especially in terms of Qa-1 subsets (because of the evident bearing of Qa-1 on suppression and immune regulation), in company with nu/nu athymic mice. Equal weight is given to (a) elucidating various bases of immunodeficiency and deviation, genetic and non-genetic, and (b) evaluating therapeutic agents - thymopoietin-TP5, ubiquitin, TCGF - in deficient, deviant and normal mice. In conjunction with tests of immune function in vitro, these immunogentic studies are intended as paradigms for investigating the nature and therapy of human disorders of immunity.

New studies, with pleiotropic-mutant W mice, aided by the Ly-5 marker system, promise to extend immunogenetic analysis to pre-lymphocytic stem sets and to identify the reputed T cell said to collaborate in erythropoiesis.

Special emphasis is placed on (a) derivation of mouse strains and stocks of Tlaa:hr genotype, to allow Qa-1 phenotyping of hr/hr and plus/hr mice, (b) determining whether hr is fully recessive, or causes a minor syndrome in heterozygotes (special relevance to man), for which purpose congenic plus/plus mice are being bred; and (c) study of ranges of normal profile variation and profile change with age in the species.

20. DEVELOPMENT OF T-LYMPHOCYTES (RABBITS, MICE)

Cantor, H. I., Sidney Farber Cancer Center Inc., *44 Binney St., Boston, Massachusetts,* 02115, United States of America

This is part of a broader project. A summary of this subproject is not available.

2. SUPPRESSOR AND HELPER T CELLS

21. SUPPRESSOR CELLS IN THE PATHOGENESIS OF CHRONIC LYMPHOCYTIC LEUKEMIA

Hersey, P., Gunz, F. W., Kanematsu Memorial Inst., Immunology Unit, *Macquarie St., Sydney, N.S.W. 2000, Australia*

OBJECTIVE: To determine whether immunodeficiency in patients with chronic lymphatic leukemia may be due to overactivity of suppressor cells of the immune response.

APPROACH: The methods used in these studies were similar to those described by Moretta et al. (1977). B lymphocytes and T lymphocyes were separated from peripheral blood of CLL patients and age matched normal controls by sheep red cell (E) rosetting procedures similar to methods described previously (Hersey et al., 1975). The B lymphocytes of the normal controls were then mixed with varying numbers of normal T lymphocytes or CLL T lymphocytes and stimulated in culture for 6 days by the polyclonal B cell activator, pokeweed mitogen. Ig classes - IgG, IgM, and IgA - that have been synthesized in the cultures over this period were measured by nephelometric methods. Normal B lymphocytes received maximal 'help' by low numbers of T lymphocytes from normal subjects but were frequently unresponsive in the presence of T cells from CLL patients. To determine whether CLL T cells were deficient in 'help' or had excessive suppressor activity, they were added in varying numbers to cultures of normal B cells and T cells at optimal ratios for Ig production. Suppressor activity of the CLL T cells was then shown by the suppression of Ig synthesis from B cells in the presence of normal T cells. This culture system also allowed the function of CLL B lymphocyes to be tested by culturing them in the presence of normal T cells. The cell separation and culture procedures are now well established in our laboratory.

PROGRESS: Our results show that in patients with untreated CLL, immunoglobulin synthesis in vitro appeared to be suppressed by their T cells. In patients with more advanced CLL who had received various forms of treatment, abnormalities in both their 'helper' T cells and B lymphocytes could be detected. No correlation was found between cell division and immunoglobulin synthesis in the cultures.

These results suggest that the underlying reason for immunodeficiency in CLL patients may change at different stages of the disease. (Text Abridged.)

BIBLIOGRAPHIC REFERENCES: Hersey, P., Edwards, A.E., and Edwards, J. Clin. Exp. Immunol., 23, 104, 1976. Moretta, L., Webb, S.R., Grossi, C.E., Lydyard, P.M., and Cooper, M.D. J. Exp., Med., 146, 184, 1977.

22. CORRELATIVE STUDIES ON THE MEMBRANE PROPERTIES AND FUNCTIONS OF LYMPHOCYTES

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We previously reported that normal as well as chronic lymphocytic leukemia (CLL) peripheral blood lymphocytes (PBL) which bear complement receptors (CRL) bind BAC (Bacillus S. typhi sensitized with IgM antibody and complement). At 37 degrees C normal CRL promptly cap adherent BAC; in contrast, CRL from untreated CLL patients cap poorly, if at all. We have now observed normalization of CLL-CRL capping within a few weeks after successful treatment of patients with cytotoxic agents. This persisted for periods of weeks to months but invariably declined to pretreatment levels prior to clinical exacerbation of the CLL. Studies are now in progress to determine whether this is, perhaps, the earliest laboratory accompaniment of clinical deterioration in such patients.

Studies of BAC prepared under different conditions of complement fixation reveal that a) C3d, rather than C3b, is the major C3 antigen displayed by BAC as routinely prepared in our laboratory; b) however, significant BAC3b, in addition to BAC3d, may be generated simply by shortening the exposure of BA to fresh mouse serum thereby limiting the exposure of BAC3b to the serum C3b INA (inactivator). In contrast to normal B lymphocytes which bind both BAC3d and BAC3b, malignant lymphocytes freshly obtained from CLL patients or grown in long-term culture (eg. Raji, Daudi) bear primarily C3d receptors.

Bone marrow culture studies of a patient with a chronic T cell lymphoproliferative disorder and profound neutropenia revealed normal growth of granulocyte/monocyte colonies in vitro. The patient's peripheral blood lymphocytes, but not monocytes, elaborated colony-stimulating activity (CSA) when appropriately activated. We have initiated studies in an effort to determine whether the monocyte abnormality is intrinsic (primary) or secondary to the lymphocyte abnormalities present in this patient (excessive suppressor T cells).

23. CONCANAVALIN A STIMULATED SUPPRESSOR LYMPHOCYTES IN HODGKIN'S AND NON-HODGKIN'S LYMPHOMA PATIENTS

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OBJECTIVES: The functionality of T suppressor populations in both Hodgkin's and non-Hodgkin's lymphoma patients will be studied. All cases will be previously untreated patients. After chemotherapy, the patients will be re-studied in order to determine if the T lymphocyte suppressor populations differ functionally before and after treatment.

METHODS: Lymphocytes from peripheral blood will be obtained and either preincubated with concanavalin A (Con A) or not. Cultures of Con A stimulated lymphocytes (and respective controls without Con A induced cells) and autologous lymphoid cells will then be stimulated with PHA and Con A, and T suppressor cell inhibition activity will be evaluated.

24. SUPPRESSOR T-CELLS IN MALIGNANT LYMPHOMA

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The objective of this study is the delineation and characterization of suppressor T cell activity in patients with Hodgkin's disease and non-Hodgkin's lymphomas. The impaired function of lymphocytes in these patients has been the subject of many studies. Recent improvements in methodology have suggested possible means for the separation and study of several subpopulations of T lymphocytes. Cells which specifically suppress effector lymphocytes have been defined in animal systems and in some human disorders. Our initial studies suggest that a subpopulation of lymphocytes from patients with Hodgkin's disease and non-Hodgkin's lymphoma may cause inhibition of the cytolytic activity of T cells directed against lymphocyte target cells. This study proposes to expand on these initial findings and to evaluate the role of suppressor T cells in malignant lymphomas by studying the in vitro effect of patient lymphocyte subpopulations, depleted of or enriched with suppressor T cells, on effector T lymphocyte function.

25. SUPPRESSOR CELL INDUCTION IN LYMPHOMA

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Patients with lymphoma have been shown to exhibit immunosuppression which has been attributed to the presence of suppressor monocytes and T-cells. Based on clinical observations and our recent studies we have developed the following hypothesis to explain the induction of suppressor monocytes and T-cells in lymphoma patients: neoplastic B-cells induce T-cell proliferation, T-suppressors and T-cell production of lymphokines. Monocyte turnover and maturation into macrophages are enhanced by the increased presence of lymphokines and suppressor monocyte-macrophages are induced. Increased levels of macrophage enzymes further enhance proliferation in this 'in vivo autologous mixed lymphocyte culture.' To test this hypothesis we will undertake the following experiments: In clinical studies: (a) The level of immunocompetence in untreated lymphoma patients will be determined and it will be correlated with the presence of suppressor monocyte and T-cells; (b) the capacity of neoplastic B-cells and circulating B-cells to induce proliferation and suppressor T-cells will be compared; (c) membrane structure and antigens of neoplastic and circulating B-cells will be compared; (d) the capacity of cultured macrophages from lymphoma patients to produce and secrete enzymes will be measured. In basic studies: (a) The capacity of monocyte-macrophage, and monocyte-macrophage enzymes to alter suppressor T-cell specificity in mixed lymphocyte cultures will be examined; (b) the capacity of macrophage enzymes to alter B-cell surface structure and antigenicity will be examined.

26. IMMUNE CELL INTERACTIONS IN LEUKEMIA, LYMPHOMA AND AUTOIMMUNE DISORDERS

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Autologous mixed lymphocyte culture (AMLC) reactions occur between T cells (responders) and B cells (stimulators) of all normal individuals and all normal mouse strains so far tested. AMLC reactions result in generation of suppressor T cells. AMLC reactions are deficient in lymphoproliferative and autoimmune disorders in humans and in the autoimmune mouse strain, NZB. My objectives are to determine the nature of the regulation of the immune system exerted by AMLC activated T cells and to determine the relationship of deficient AMLC reactions to the pathogenesis of lymphoproliferative and autoimmune disorders of humans and mice. The objectives will be accomplished by 1) analyzing the effect of AMLC activated cells on in vitro immune responses, 2) determining whether the defects in AMCL reactions found in lymphoproliferative and autoimmune disorders are at the T cell or B cell level, 3) characterizing the cell surface markers of the responding and stimulating cells, 4) studying restrictions on the system imposed by the major histocompatibility complex, 5) determining the relationship of deficient AMLC reactions to the occurrence of autoantibodies in autoimmune disorders, and 6) performing genetic analysis of deficient AMLC and autoantibody formation in NZB x C58 recombinant-inbred lines of mice.

27. EFFECTOR AND SUPPRESSOR CELL FUNCTION IN HUMAN LUNG NEOPLASIA

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The objective of the current project is to investigate cytotoxic and memory lymphocyte responses to human lung malignancies and factors which compromise their effective expression. Cytotoxicity is monitored in a 4 hour 51Cr release assay against targets freshly isolated from the tumor or normal lung specimen by separation on discontinuous Ficoll-hypaque gradients. The MLTI response and in vitro generation (IVG) of cytotoxic effectors upon cocultivation of blood lymphocytes and autologous tumor indicate memory potential. In approximately 30% of lung cancer patients significant killing of autologous tumor cells can be measured under conditions where normal lung, allogeneic lung tumors or tumors arising at other sites are refractory. Freshly isolated targets show only minimal sensitivity to natural killers. MLTI responses and IVG occur more frequently (60%) possibly revealing a depression of effector reactivity. Tumor draining lymph nodes show reactivity at comparable rates to PBL but tumor infiltrating lymphocytes are only rarely reactive (less than 5%). These latter cells have powerful suppressive activity of PAA transformation and MLTI, with SRBC rosetting or nylon-column passed cells having the greater suppressor role. In PBL both monocytic (adherent carrageenan, silica and indomethacin sensitive) and T cells can function as suppressors. The effect of all these elements on IVG will be assessed.

28. SUPPRESSOR CELL ACTIVITY IN BLADDER CANCER PATIENTS

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We developed a reproducible assay for the measurement of concanavalin A (Con A)-inducible suppressor cells. Using this assay system we studied suppressor cell activity of peripheral blood lymphocytes (PBL) from urologic cancer and control patients. Although lymphocyte proliferation to Con A was depressed in many of the cancer patients, no difference in suppressor cell activity between cancer and control patients was observed. These results suggest that suppressor cells of the type induced by Con A are not responsible for the immunosuppression observed in urologic cancer patients.

We found that protein A from Staphylococcus aureus (SpA) induced Type II interferon production in human PBL. Maximum interferon production was observed 24 hours after treatment with SpA. Moreover, unlike other mitogen interferon inducers, induction of interferon was enhanced by macrophage depletion. Cell fractionation studies showed that the interferon-producing cell was a nonadherent Fc receptor-positive lymphocyte.

Studies on the effects of Type II interferon on natural killing (NK) and antibody-dependent cell-mediated cytotoxicity (ADCC) suggest that both NK and ADCC are augmented by Type II IF treatment.

29. IMMUNOBIOLOGICAL INVESTIGATION OF SUPPRESSOR LYMPHOCYTES DERIVED FROM HUMAN FETUSES AND INFANTS

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We have previously shown that lymphocytes from the human newborn regularly abrogate in vitro the proliferation of adults' lymphocytes stimulated by alloantigens or by PHA. This was borne out by experiments using two-way mixed lymphocyte culture (MLC) and sex chromosomes as markers for dividing cells. The suppressor effect is apparently an exclusive function of the baby's T lymphocytes. The baby's lymphocytes do not kill the maternal lymphocytes judging from cytotoxicity assays. Further, the suppressor activity seems to be mediated - at least partly - by a low molecular weight substance(s) released by the newborn's lymphocytes. We are currently investigating the ontogeny and postnatal persistence of the suppressor cell activity as well as the chemical nature of the soluble suppressor substance. In addition, the suppressive effect, if any, on the proliferation of tumor cells in vitro is currently investigated.

30. THE SUPPRESSOR-CELL NETWORK IN CANCER - ROLE OF THE THYMUS GLAND AND IMMUNOSUPPRESSION IN CANCER PATIENTS

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OBJECTIVE: (1) Role of the thymus in human oncogenesis: correlation of thymic pathology and cancer risk. (2) Immunosuppressive activity of lymphoid cells present in neoplastic pleural and peritoneal effusions.

APPROACH: (1) Identification and functional characterization of lymphocyte subpopulations in normal, neoplastic and hyperplastic human thymus glands. Lymphocyte subsets and cellular immune function in myasthenic patients before and after thymectomy. (2) Identification and functional characterization of lymphoid subsets in human neoplastic effusions.

PROGRESS: We published data concerning: Subpopulations of T lymphocytes in myasthenia gravis patients (Piantelli et al., Clin. Exp. Immunol. 36, 85, 1979), subpopulations of lymphocytes in human thymomas (Lauriola et al., Clin. Exp. Immunol. 37, 502, 1979), Fc receptor for IgM: factors influencing detection on human T lymphocytes (Carbone et al., J. Immunol. Method 29, 245, 1979).

31. SPECIFIC TOXICITY OF EHNA FOR HUMAN SUPPRESSOR CELLS

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The purpose of this study is to investigate the ability of an adenosine deaminase inhibitor (EHNA) in the presence of the purines adenosine and deoxyadenosine to specifically kill human T-suppressor cells in vitro. Our preliminary studies indicate that purines and EHNA are selectively cytotoxic to human T-suppressor cells which function in the primary (IgM) humoral immune response and that removal of the suppressor population results in greatly increased antibody production in vitro. The specific objectives of this study are: (1) The characterization of the most effective concentrations of EHNA and purines for selective cytotoxicity of T-suppressor cells for the primary hurmoral immune response. (2) Characterization of the most effective concentration of EHNA and purines for selective cytotoxicity of T-suppressor cells which inhibit the conduct of T-cell-mediated MLR. (3) Characterization of the kinetics of suppressor T-cell induction in the humoral immune response and of T-suppressor cell induction in the T-cell-mediated reaction. (4) Characterization of T-suppressor cell specificity in the humoral response and suppressor cell specificity in the T-cell-mediated MLR response. (5) Characterization of static (antigen and mitogen free) and antigen or mitogen induced suppressor cell cytotoxicity profiles. (6) Quantitation of the level of adenosine deaminase in subpopulations of human lymphocytes enriched with and depleted of suppressor cells.

32. SUPPRESSOR CELLS IN CANCER AND IMMUNODEFICIENCIES

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Immunodeficiency states, both cell mediated and humoral, may be a primary pathological manifestation or they may occur secondary to other diseases such as cancer. This project will investigate the role of suppressor cells in the pathogenesis of immunodeficiency states. We have demonstrated by co-cultivation techniques leukocyte subpopulations capable of suppressing B cell and/or T cell associated functions in the peripheral blood of immunodeficient patients and have correlated these observations with the clinical status of the patients under investigation. We propose to isolate from the patients suppressor cells described by selective rosetting techniques, column chromatography, adsorption on solid state matrices, density gradient and sedimentation velocity separations and the use of specific antisera. Our studies and those of others have already demonstrated the selective radiosensitivity of naturally occurring human suppressor cells. We shall further characterize these cells on the basis of surface marker properties. Particular attention will be directed to the newly described T cell subpopulations such as T gamma and T mu lymphocytes and their roles in immunoregulation. We are presently examining the immunoregulatory role of prostaglandins. Additional efforts will be directed at the biochemical isolation and characterization of other human soluble suppressor substances. Studies are presently underway in our laboratories using Marbrooke flasks to separate suppressor cells from normal donor cells by membrane filters, allowing only soluble factors to pass between the separated populations. Thus the characterization of a cellular and biochemical mechanism leading to immunodeficiency would be the first step in developing new therapeutic modalities for these conditions.

33. SUPPRESSOR CELLS IN CURED CANCER PATIENTS

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OBJECTIVES: This study will answer the following questions: 1. Is the presence of suppressor cells predictive of response to chemotherapy? 2. Do suppressor cells predict tumor recurrence following a 'curative' maneuver such as ablative surgery?

METHODOLOGY: Peripheral blood mononuclear cells are isolated by density gradient centrifugation. T-suppressor cells are quantitated in two ways: by induction with concanavalin A (Con A) and by indirect immunofluorescence using OKT8, a monoclonal antibody directed against T-suppressor cells. Monocyte suppressor cells are analyzed in two ways: by depletion on plastic culture vessels and by 24-hour precultivation prior to stimulation with mitogen.

RESEARCH PLAN: Patients are studied prior to therapy and at trimonthly intervals thereafter for period of one (1) year.

34. SUPPRESSOR CELL IN ONCOLOGY PATIENTS

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This is part of a broader project. A summary of this subproject is not available.

35. HLA RESTRICTED SUPPRESSOR T CELLS OF MIXED LYMPHOCYTES

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We have described suppression of the mixed lymphocyte reaction in humans by thymus-derived T cells and soluble T cell derived factors. Such suppression is highly specific for HLA-D products on MLR responder lymphocytes. In the past year we have demonstrated that MLR suppressor cells are rare in healthy individuals but common in patients with Hodgkin's disease, a disorder characterized by diminished cell-mediated immunity. Surprisingly, MLR suppressor cell activity is increased following treatment with lymphoid irradiation. In addition, we have discovered that the autologous mixed lymphocyte reaction is markedly diminished in virtually all patients with Hodgkin's disease, a finding which is even more dramatic than the presence of suppressor cells. In the past year we have succeeded in producing a monoclonal xenoantibody to a human T cell subset, but MLR suppressor T cells are not included in this subset.

The goals for the coming year include: 1. Extended studies of the possible role, in vivo, of suppressor T cells of the MLR and their possible induction by lymphoid irradiation; 2. analysis of the profound effect of autologous MLR in Hodgkin's disease; 3. continued production of monoclonal antibodies specific for human T cell subsets, including antibodies specific for MLR suppressor T cells; 4. further definition of the genetic restriction between suppressor cells and their target responder cells; 5. attempted growth of T cell clones with specific functions, including MLR suppression; and 6. initiation of biochemical analysis of suppressor T cell factors.

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36. HISTAMINE H2 RECEPTOR T-SUPPRESSOR CELLS IN CANCER

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Suppressor T-cells play an important role in the biology of human tumors. The focus of the work we propose is the suppressor T-cell subset which is characterized by the possession of a surface receptor for histamine of the H2 type (H2R-positive). Previously we have studied this cell from biochemical and immunological perspectives. This has included the solubilization, characterization and purification of specific H1 an H2 receptors from its surface. In addition, we have demonstrated that the immature percursor of this cell requires thymic stimulation to express the H2 receptor, and have partially purified a factor from calf thymus which specifically induces the H2 receptor. Furthermore, we have shown that this cell can be activated by histamine to become a functional suppressor cell. This effect of histamine is mediated through the H2 site as evidenced by the ability of cimetidine (an H2 antagonist) to block it. We now propose a detailed study of the relationship of this specific suppressor cell to malignant disease. In order to do so we will characterize and quantitate histamine receptors on the T-cells of patients and animals with malignant tumors; determine the effect of histamine and its antagonists/agonists on the functional immunological activity of the H2R-positive cell including lymphocyte-mediated tumor cytotoxicity and suppression; determine if histamine and its antagonists/agonists play a role in the therapy of cancer based on studies of their effect on suppressor cells in an animal model; and determine the role that serum histamine may have in the pathophysiology of malignancies. We also will study the plasma and serum of patients with malignancies for the presence of a circulating factor, analogous to that which we have isolated from calf thymus, which induces histamine H2 receptors on precursor T-cells.

37. IDIOTYPE-SPECIFIC SUPPRESSOR T-CELLS AND THEIR RECEPTORS

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The objective of this research project is to explore the mechanism by which a specific thymus-dependent (T) lymphocyte modulates the immune response in a negative regulatory way and to study specific antigen-binding receptors on these T-lymphocytes. We propose to study lymphocytes which suppress two different clones of antibody-producing cells that respond to phosphorylcholine. The thesis is that the suppressor cells are T cells and that they interact with a clone by recognizing individual antigenic determinants (idiotypes) on the Ig receptors of these clones. Suppressor cells specific for two different idiotypes in the multiclonal response will be generated, isolated and characterized with respect to cell surface markers and specificity of activity for the test idiotype. Subsequent experiments will investigate the role these specific suppressors play in regulating B cell proliferation and maturation and/or T-T interactions. The research proposes to isolate and characterize the recognition unit on these suppressor lymphocytes. Analysis of these parameters will depend on separating subpopulations of lymphocytes with specific antisera and studying selected mixtures of different lymphocytes and macrophages in in vivo adoptive transfer experiments or in in vitro short-term cultures. Suppression will be monitored by measuring proliferation of B-lymphocytes bearing the idiotype and their Ig products. Somatic cell hybrids of an idiotype-specific suppressor lymphocytes and a tumor cell will be prepared in order to generate large numbers of homogeneous cells bearing characteristics of the suppressor studied. These studies should provide basic information about one aspect of how lymphocytes regulate immune responses and, we hope, give us information on the structure and function of the antigen recognition receptor on one type of T-lymphocyte.

38. MECHANISMS OF ACTIVATION OF SUPPRESSOR CELLS BY SOLID TUMORS

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The working hypothesis of this proposal is that tumor cells release factors which intervene in normal immunoregulatory pathways, directly activate non-specific suppressor cells, and thus contribute to the anergy associated with progressive tumor growth. The major objective of the proposed investigation is to establish an in vitro model system in which the mechanism of the lymphoid cell interactions involved in the activation of suppressor cells may be clarified. This will be accomplished by culturing normal spleen and tumor cells in separate compartments of Marbrook vessels separated by membranes of various pore sizes. Lymphoid cells will then be removed and assayed for the ability to suppress the generation of antibody forming cells.

Specific objectives will be to determine a) whether tumor cells, and not only passenger lymphocytes, produce factors which induce suppressor cells, b) the types of lymphocytes which are precursors of suppressor cells, c) the cell surface antigens (particularly Ly antigens) present on precursor or activated suppressor cells, d) the classes of lymphocytes suppressed, e) the effect of suppressor cells on cellular immune responses or DNA synthesis, and f) the effect of adding suppressor cells at different stages of differentiation of effector mechanisms.

This model system will provide a means of studying in detail one of the possible mechanisms of induction of suppressor cells in tumor-bearers. It is hoped that this work will provide information about the mechanisms of generalized immunosuppression in cancer patients and will ultimately provide a system in which immunological and pharmacological agents capable of interfering with suppressor cell activation or activity may be discovered.

39. REGULATION OF THE IMMUNE RESPONSE BY ANTIBODY, ANTIGEN OR SUPPRESSOR CELLS

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OBJECTIVE: To determine regulatory mechanisms and their relevance as they occur in immune responses against xenogeneic, allogeneic, modified syngeneic and tumor antigen.

APPROACH: Search for evidence and degree of end product feedback be it by antibody or cells. The assumption is that end products can regulate immune responses and the regulatory ability of such end products is studied in various model systems.

PROGRESS: Antibody feedback requires the antigen binding site, but also the Fc portion. The Fc portion of antibody is required as an inactivated signal directed at responding B cells. The requirement for the Fc portion of antibody is independent of its role in excretory phenomena. T cells and T cell products may interfere with this antibody feedback while other cells are activated by suppressive antigen antibody complexes to act as effector mechanisms in such feedback phenomena.

Cytotoxic cells formed in an in vitro cell mediated immune response will suppress a second primary cell mediated immune response. The target for this suppressive activity is the responder cells in the second culture system indicating that such suppressor phenomenon would have a direct regulatory effect on the responding cells rather than represent an epiphenomenon of antigen elimination.

40. INDUCTION OF TOLERANCE AND SUPPRESSOR CELLS IN VITRO

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We intend to study the induction of specific immunological unresponsiveness in cultures of lymphoid cells from rabbits and the transfer of such unresponsiveness to other cultures by suppressor cells. The model is a primary in vitro antibody response to solubilized phage antigens which is free of demonstrable requirements for help by either macrophages or T-lymphocytes and which is entirely IgM. We shall examine two putative mechanisms leading to unresponsiveness, namely B-cell receptor blockade and the induction of T-suppressor activity. Hypotheses to be tested include one that visualizes immunity and tolerance induction as events that occur simultaneously in response to a wide dose of antigen concentrations and another which considers B cells with blocked receptors a particularly effective inducer of specific T-suppressor cells. The in vitro system lends itself particularly well to the cell separation and reconstitution experiments that need to be done for a better insight into immunoregulatory processes which, in turn, will be needed for improvements in the understanding and practice of immunological manipulations in allergy, autoimmune disease and cancer.

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41. STUDY OF THE REGULATION OF THE HUMORAL ANTITUMOR IMMUNE RESPONSE

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OBJECTIVE: To verify the generality of the previously observed phenomenon of a possible interference of T-suppressor cells in the production of natural antitumor antibodies in some murine strains and to elucidate the importance of T-suppressor activity in the antitumor responses induced by immunization.

APPROACH: Mice will be deprived of T-suppressor cells: 1) by chemicals, such as colchicine and cyclophosphamide, which are known to block the suppressor cells at the time of their development soon after the immunizing treatment; 2) by antisera directed against IJ region of the MHC, specifically directed against the T-suppressor cells. Lymphoid cell populations will be deprived of the suppressor cells by passage on histamine columns and injected in immunodepressed mice. The immune response of the various groups of so treated animals will be studied by in vitro cytotoxicity assay and by in vivo resistance to tumor growth.

PROGRESS: The inoculum of colchicine or cyclophosphamide together with tumor cells induced an antitumor antibody production which did not take place when the tumor cells were injected without the chemicals.

42. CONTROL OF IMMUNOSUPPRESSOR CELL ACTIVATION FUNCTION

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Treating mice with radioactive strontium results in local irradiation of bone marrow and bone and the destruction of cellular elements which depend upon the micro-environment of the bone marrow for differentiation. Although T and B cell immunity and other hemopoietic cell functions appear to be relatively normal in these animals, a number of host resistance phenomena are markedly altered. Many of these normal host resistance events appear to be the result of alterations in the control of immune reactions. Recent studies in our laboratory have demonstrated that suppressor cells obtained from animals treated with 89Sr are easily activated and become immunosuppressive in times and under conditions when normal immunosuppressive activity does occur. It is our intention to determine what mechanism of control is altered by radiation of the bone marrow micro-environment. We will determine if there is cellular involvement in this loss of control and whether there are humoral immunoregulatory factors which have become altered. If regulatory cells are involved we will try to determine what type or types of cells they are. It will be determined whether immunosuppressive cells become activated in response to a positive stimulus or rather an escape from a normally inhibitory control. Assay methods will include in vitro primary antibody response using the Mishell-Dutton assay system, adoptive transfer in inbred mice, and proliferative responses to mitogens.

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43. REGULATION OF NORMAL AND ANTI-TUMOR IMMUNE RESPONSES

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The activity of suppressor cells in regulating immune responses is now widely accepted, but the ability to control this regulation awaits a better understanding of the nature and action of these cells. Our previous work has defined a feedback suppression system in mice in which a subset of unprimed cells is stimulated in vitro to act as suppressor cells in the presence of immune spleen cells. The subset of T cells which can be activated has been characterized and it is now proposed to isolate and characterize, and determine the mechanism of suppression of the feedback activated suppressor cells. These activated cells will be isolated from in vitro activated normal spleen cells or from immunized mice. The timing of their activation in vivo and the origin of the precursor cells will also be investigated. In vitro activated suppressor cells will be used to determine the target cell of this feedback suppression, the function which is suppressed, and the affinity of the suppressive factor produced by the activated suppressor cell for antigen or macrophages. The comparative activities of this feedback regulatory pathway in normal and tumor bearing animals will also be investigated in both general immune responses and specific anti-tumor responses using tumor systems which induce primarily humoral or primarily cell-mediated responses.

44. REGULATOR LYMPHOCYTES OF DYSGAMMAGLOBULINEMIC CHICKS

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The research proposed for the next year is aimed at understanding the mode(s) of action of chicken thymus derived suppressor lymphocytes (Ts). Several questions are currently being asked: a) What are the target antigens that are recognized by Ts? b) When during ontogeny do the Ts target antigens become expressed? c) What are the histocompatibility requirements for Ts activity? d) Are viable Ts required for the suppression of immunoglobulin synthesis or can soluble Ts-derived factors produce suppression? e) What is the functional capability of the B-lymphocytes present in recipients made agammaglobulinemic by transplantation of Ts cells?

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45. LYMPHOID CELL INTERACTIONS IN MALIGNANCY

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The purpose of the present study is to better define the roles of lymphoid cell subtypes in tumor immunity and to determine which cellular interactions lead most directly to tumor destruction. Procedures will be adapted or developed to increase the populations of cells that interact with the tumor in a detrimental fashion and to decrease the populations of cells that interfere with tumor destruction or in any way protect the tumor. These include cells which are directly inhibitory or which inhibit indirectly by producing suppressor factors. Preliminary work has shown that spleen cells from tumor-bearing mice release a soluble factor which causes immunosuppression. The suppressor factor is non-antigen specific and is produced by T-cells. A primary goal is to further characterize the chemical and biological properties of this factor.

46. CELL INTERACTIONS IN TUMOR IMMUNITY

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We propose to investigate the development of suppressor cell activity in mice bearing allogeneic, xenogeneic and syngeneic tumors. The suppressor cells inhibit proliferation of normal mouse spleen cells in mixed lymphocyte cultures (MLC). Tumors of various strains of origin and cell type, both recently induced and long established, will be tested for their ability to activate suppressor cells. Cytotoxic and suppressor T-cells will be separated on the basis of adsorption and possibly on the basis of XR and cortisone sensitivity. The metabolic requirements of suppressor cells and the nature of the factor(s) they produce will be investigated.

47. LYMPHOCYTE INTERACTIONS, RECEPTORS AND FUNCTIONS

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A B cell leukemia (L2C) of inbred strain 2 guinea pigs and a new myelogenous leukemia (G-13) of inbred strain 13 guinea pigs are being investigated as models of human leukemia. The chemical nature of the tumor specific transplantation antigen (TSTA) of the L2C leukemia is being investigated by immunization protection tests in syngeneic animals. The TSTA of the L2C leukemia is unusual in that it is of low molecular weight and is extremely resistant to heating. The G-13 leukemia is being examined for the presence of a TSTA and cell surface markers. As in the human, the myeloblasts of this leukemia are Ia positive. The immune functions of these cells are also being investigated.

Patients with systemic lupus erythematosus (SLE) have a defect in the development of Con A induced suppressor cells. Moreover, the sera from lupus patients (more particularly the IgM fraction) can induce suppressor cell defects in populations of normal lymphocytes. T gamma and T non-gamma cells mediate different types of suppression; certain lupus sera are specifically cytotoxic for T gamma cells. Finally T cells able to proliferate in response to autologous non-T cells act as precursors of Con A induced suppressor cells.

BIBLIOGRAPHIC REFERENCES: Gelfand, M.C., Frank, M.M., Green, I., and Shin, M.L.: Binding sites for immune complexes containing IgG in the renal interstitium of man and other species. Clinical Immunology and Immunopathology 13: 19, 1979. Nilsson, S.F., Edelson, R., Mann, D., Green, I., and Waxdal, M.J.: Concanavalin A binding proteins on the surface of human malignant and normal lymphocytes. Scand. J. Immunol. In press, 1979.

48. ADHERENT SUPPRESSOR CELLS IN TUMOR-BEARING MICE

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OBJECTIVE: To determine the cellular/molecular interactions leading to suppression of recognitive immunity by syngeneic tumors in mice.

Inoculation of BALB/c or (BALB/c x C57BL/6)F1 mice with syngeneic murine sarcoma virus-transformed tumor cells induced a population of adherent splenic cells which non-specifically suppressed the allogeneic response in mixed leukocyte culture (MLC). Removal of the adherent cells by passage of spleen cells over columns of Sephadex G-10 beads significantly increased their capacity to respond in MLC. Experiments were carried out to determine whether the suppressive adherent cells also suppressed the cell-mediated lympholysis (CML), the effector phase of this immune response. Tumor-bearing mice demonstrated significantly suppressed CML responses which could be returned to normal by filtration of the spleen cells through Sephadex G-10 beads to remove adherent cells. Experiments have also been carried out to determine whether T cells are required for the induction of suppressive adherent cells. Since they could be induced in nude mice by inoculation with syngeneic tumor cells, suppressive adherent cells do not appear to require T cells for their induction.

49. THE CHARACTERIZATION OF A5A IDIOTYPIC SUPPRESSOR CELLS

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The objective of this proposal is to characterize the T cell receptor on the A5A idiotypic suppressor T cell described by Dr. Klaus Eichmann.

The proposed study will attempt to establish cultures of A5A plus enriched suppressor T cells by induction of suppressor cells with guinea pig IgG2 anti-A5A-idiotype serum. Suppressor cells will be enriched by using plates or columns coated with anti-idiotype serum as an immune absorbant. Long-term cultures of these cells will be established using cell free supernatants from Con A stimulated spleen cell cultures as a supportive medium. Cloning of these established suppressor cell cultures will be attempted.

The cultured suppressor populations and clones will be functionally analyzed as to enrichment of suppressor activity, cell surface markers, and the specificity of these cells for antigen and the A5A idiotype using in vitro microcultures and PFC assays.

Finally selected suppressor cell lines will be characterized as to the nature of their cell surface receptor using radiolabeling and immunoprecipitation techniques to isolate the receptor, and chromatographic techniques to study its properties.

50. LYMPHOCYTE ALLOGENEIC INHIBITION AND TUMOR IMMUNITY

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We will continue our studies on the relationship between 'specific blocking factors' (SBF), T suppressor cells, and effector cells in tumor immunity. Our working hypothesis is that SBF, in the form of tumor antigen and antigen-antibody complexes, inhibit cell-mediated anti-tumor immunity by activating T suppressor cells which then prevent the establishment of proper T cell help. A third SBF, a protein with a molecular weight of 56,000, is believed to be a T suppressor cell product whose role in suppression is being investigated. We have recently established T-T hybridomas, using T cells from BALB/c mice with MCA-induced sarcomas and hybridizing them with cells from the BW5147 AKR thymoma line. We have shown that a clone of such a hybridoma forms a specific suppressor factor that is active in vivo and in vitro. We are expanding this work by trying to establish suppressor factor producing hybridomas in the MSV sarcoma system, working with an A x BALB/c F1 hybrid mice. The suppressor factors will be characterized with respect to expression of IJ antigens and the presence of idiotypic determinants. Attempts are being made to develop radioimmunoassays for the different types of SBF.

51. SUPPRESSOR CELL INDUCTION BY RETICULUM CELL SARCOMA (MICE)

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This is part of a broader project. A summary of this subproject is not available.

52. IMMUNOLOGY OF HUMAN LYMPHOID TUMORS OF B-CELL ORIGIN

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The research goals for the coming year are as follows: 1) To study the suppressor and helper function of isolated non-malignant T lymphocytes from patients with newly diagnosed non-Hodgkin's lymphomas predominantly of B cell types. This study will comprise Ig's production in vitro by PWM-stimulated normal B lymphocytes and, if available, by autologous and allogeneic malignant B lymphocytes. The proliferative response of patients' T-lymphocytes to stimulation with various mitogens will be also studied by 3H-thymidine incorporation in vitro. 2) The study of suppressor and/or helper function of malignant T lymphocytes from cases with T-cell malignant lymphomas and leukemias will continue as in previous years. We will use allogeneic non-malignant B lymphocytes stimulated by PWM as the indicator system. 3) The relations of the expression of Fc gamma and Fc micron receptors on T and B lymphocytes with their suppressor and helper function will be studied in mitogen-induced proliferation of autologous lymphocytes. 4) We will continue to identify the phenotypes of malignant lymphoma cells which do or do not respond to glucocorticoids in vitro. This is extremely important since we have shown recently that there is a good correlation between the in vitro tests and the patient's response to glucocorticoid therapy.

BIBLIOGRAPHIC REFERENCES: Bloomfield, C.D.; Gajl-Peczalska, K.J.; Frizzera, G.; Kersey, J.H.; Goldman, A.I. Clinical utility of lymphocyte surface markers combined with the Lukes-Collins histologic classification in adult lymphoma. N. Eng. J. Med. 301, 512-518, 1979. Hurwitz, R.; Hozier, J.; Le Bien, T.; Minowada, J.; Gajl-Peczalska, K.; Kubonishi, I. and Kersey, J.H. Characterization of a leukemic cell line of the pre-B phenotype. Int. J. Cancer 23, 174-180, 1979.

53. IMMUNOREGULATORY CELLS IN HUMAN NEOPLASIA

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The purpose of this project is to analyze the cellular control of immune function in normal individuals and in certain patients with neoplastic disease. We are currently determining the helper and suppressor activity of neoplastic T cells from patients with acute lymphoblastic leukemia and cutaneous T-cell lymphomas. We are also studying abnormalities of regulatory monocytes and T-cells from patients with multiple myeloma and mice bearing plasmacytomas. Finally, we are characterizing the capacity of antibodies directed against surface membrane products of immune respone genes to inhibit human immune reactions by activating regulatory T cells.

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54. COMPARISON OF HELPER AND SUPPRESSOR T CELL RECEPTORS

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An imbalance in T helper and suppressor cell activity has been associated with certain disease states such as tumor growth and hypogammaglobulinemia. Understanding the mechanisms involved in regulating immune responses is important for treatment and/or prevention of diseases.

Simulation of lymphocytes is thought to occur via surface receptors. Although antigen binding receptors on B lymphocytes have been well characterized, T receptors are difficult to isolate and thus have not been well defined. Helper and suppressor T cells can be induced by stimulating mice with the IgG1 and IgG2 fractions, respectively, of anti-idiotype sera. Hybrid cell lines will be made by fusing specifically sensitized splenic T cells with T lymphoma cells. T helper and suppressor cell receptor materials will be recovered from the hybrid cells and compared in terms of their ability to bind antigen and to inhibit idiotype anti-idiotype binding. Establishing hybrids bearing T cell receptors will provide a source of large amounts of receptor material which can then be studied biochemically.

55. ROLE OF CELL-MEDIATED IMMUNE RESPONSES IN ANTI-TUMOR IMMUNITY

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OBJECTIVE: To analyze the regulatory mechanisms which control the generation of suppressor cells for cytotoxic T cell responses. This has the ultimate aim of determining whether or not the apparent inefficiency of T cell-mediated immunity in combatting neoplasia is due to pre-existing inhibitory networks which, if perturbed, would allow effective anti-tumor CMI.

APPROACH: 1. Analysis of both the helper and inhibitory cells which may participate in the induction of suppressor T cells. 2. Analysis of the regulatory controls operative in ongoing environmentally primed responses to exogenous and endogenous antigens. 3. Attempt to enhance cytotoxic responses to tumor associated antigens by provision of both artificial determinants and helper T cells primed to those determinants.

56. ANALYSIS OF THE CELLULAR BASIS OF HELPER AND SUPPRESSOR FUNCTION

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This is part of a broader project. A summary of this subproject is not available.

57. LYMPHOCYTE SENSITIZATION TO TUMOR ANTIGENS IN VITRO

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Several areas of continued research on the sensitization of human lymphoid cells to antigens on melanoma cells are proposed. Lymphocyte subpopulations will be tested for their ability to be specifically sensitized to autochthonous melanoma cells, and studied with regard to the expression of natural killer (NK) cytotoxicity. A system of T-cell 'help' has been investigated, and will now be applied in an attempt to augment the development of cytotoxic effector cells. Secondary sensitization with soluble tumor antigens after in vivo or in vitro priming will be investigated. The sensitization system will be used to study the expression of various melanoma-associated antigens and histocompatibility restrictions on the cytotoxic response.

BIBLIOGRAPHIC REFERENCES: Seeley, J.K., Masucci, G., Poros, A., Klein, E., and Golub, S.H.: Studies on cytotoxicity generated in human mixed lymphocyte cultures. II. Anti-K562 effectors are distinct from allospecific CTL and can be generated from NK-depleted T cells. J. Immunol., 123:1303-1311, 1979. Golightly, M.G., and Golub, S.H.: The in vitro culture characteristics and requirements for expression of receptors for IgM on human peripheral blood lymphocytes. Clin. Exp. Imm., 39:222-232, 1980.

58. KILLER ASSISTING FACTOR IN CELL MEDIATED LYMPHOLYSIS

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The object of this grant is to determine the immunoregulatory function(s) of a soluble helper factor(s), which is involved in the generation of cytotoxic T (thymus derived) lymphocytes that are directed against allogeneic cells and syngeneic tumors. To accomplish this, we are seeking to identify certain biological, biochemical, and functional properties of this helper factor, and to determine its relationship to other immunoregulatory molecules (i.e., T cell growth factor, lymphocyte activating factor, interferon).

59. GENETIC CONTROL OF THE IMMUNE RESPONSE IN VITRO

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Requirement for self-recognition by T-helper cells and B cells has been analyzed in the in vitro primary antibody responses to the thymic dependent antigen TNP-KLH and the thymic independent antigen TNP-Ficoll. T cells obtained from A-produces-AxB, AxB-produces-A, and A-produces-B radiation bone marrow chimeras were tolerant to both A and B MHC determinants but could only cooperate with macrophages expressing the I-A determinants of the chimeric host, not the chimeric donor. The recognition by T-helper cells of macrophage I-A determinants was specifically blocked by anti-I-A antibodies directed at macrophage I-A determinants, suggesting that the I region gene products recognized by T-helper cells are also those detected serologically. Surprisingly, for TNP-Ficoll responses, B cells from AxB-produces-A and A-produces-B chimeras were also restricted to cooperating only with macrophages expressing MHC determinants of the chimeric host, strongly suggesting that B cell activation requires B cell recognition of macrophage MHC determinants.

BIBLIOGRAPHIC REFERENCES: Singer, A., Hathcock, K. S., and Hodes, R. J.: MHC restricted helper T cell recognition of accessory cells but not B cells. In Rosenthal, A., and Unanue, E. (Eds.): Macrophage Regulation of Immunity. Academic Press, New York, New York, pp. 153-172, 1980. Singer, A., Hathcock, K. S., and Hodes, R. J.: Cellular and genetic control of antibody responses. V. Helper T cell recognition of H-2 determinants on accessory cells but not B cells. J. Exp. Med. 149: 1208-1226, 1979.

60. CONTROL AND FUNCTION OF THYMUS DERIVED CELLS IN THE IMMUNE RESPONSE

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OBJECTIVES: To understand the differentiation and functional heterogeneity of mouse helper T cells. To investigate the role of H-2 gene products in antigen recognition by helper T cells, and in their differentiation in the thymus.

APPROACH: Murine helper T cells are primed with a number of antigens in vivo, including bone marrow chimeric mice of various origins. They are then assayed in vitro for their ability to cooperate with B cells and macrophages of various origins. If necessary, T cells from different types of mice will be combined before priming in short-term chimeric mice.

CURRENT PLANS AND PROGRESS: We have shown that at least two types of helper T cell exist in mice, with different modes of action. Only one of these types is required for B cells to respond to red blood cell bound antigens, but both are probably required for responses to polypeptide bound antigens. Our current experiments are designed to test this hypothesis.

Both these types of T cell are primed up to recognize antigen in association with I region products on macrophage surface. At the effector phase, we will investigate whether these cells recognize I region product plus antigen on different cells.

At least one of these T cells is imprinted in the thymus in such a way that it can only recognize antigen in association with I region products expressed there. We will find out which one.

61. EVALUATION OF GENETIC RESTRICTION IN THE ACTIVATION OF HELPER T-CELL FUNCTION

Sopori, M. L., University of Kentucky, School of Medicine, Dept. of Pathology, *Limestone & Euclid, Lexington, Kentucky,* 40506, United States of America

Our studies to date reveal that spleen cells from athymic (BALB/C (H-2d)) nude mice are capable of generating a primary in vitro antibody response to SRBC when provided with either H-2 syngeneic or allogeneic gamma-irradiated (2000 rad) or mitomycin c-treated T-cells from spleens or lymph nodes. However, a similar helper function, if provided by thymocytes, is strictly H-2 restricted. The experiments proposed in this project are primarily aimed at determining the nature of this apparent disparity in the expression of H-2 linked helper function between T-cells obtained from lymph nodes/spleens and those obtained from thymocytes. The precise questions posed towards this goal are: (a) Which H-2 loci are involved in the H-2 linked 'help'; (b) possible role of macrophages in the expression of H-2 restricted help; (c) population of thymocytes that exhibit the restriction; (d) form in which 'help' is made available (e.g. cell-to-cell contact or soluble mediators) both in restricted and in unrestricted helper function; and (e) use of metabolic inhibitors in conjuction with gamma-irradiation to evaluate early molecular events leading to the generation of helper signal. Understanding the nature of helper signal will eventually facilitate the generation of CTLs directed against the CD determinants of sygeneic tumor cells.

3. CYTOTOXIC T CELLS

62. SPECIFIC CYTOTOXICITY CAUSED BY T-LYMPHOCYTES FROM PATIENTS WITH EBV-RELATED DISEASES, MAINLY IM (ABBREV)

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OBJECTIVE: We have found killer T-cells with EBV-related specificity in infectious mononucleosis (IM) cases (EBV-caused). The antigen responsible for target sensitivity has been termed LYDMA. The purpose of this investigation is to further define the role of the anti-LYDMA cytotoxic cells in limiting the EBV infection and protecting against possible leukemialike spread of the persistent EBV-transformed cells.

APPROACH: We intend to study primary EBV infections which develop IM or have no characteristic symptoms, selected initially as susceptible IM contacts, and compare development of LYDMA-specific cytotoxicity, serology and clinical findings. Further characterization of LYDMA, the killer cells, as well as investigation of a possible role of 'syngeneic restriction,' will be attempted, using biochemical, serological and cellular techniques. Present evidence indicates that 'syngeneic restriction' does not play a major role in acute IM.

63. FUNCTIONS OF ATYPICAL LYMPHOCYTES

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Infectious mononucleosis (IM) is a lymphoproliferative syndrome characterized by an atypical lymphocytosis. Most cases are caused by the Epstein-Barr virus (EBV), however a smaller proportion results from infection with cytomegalovirus (CMV) and mononucleosis can be induced, albeit rarely, by adenovirus, rubella and Toxoplasma gondii. The pathogenesis of the disease caused by EBV has received most study. EBV infects the B cell and the atypical lymphocytes include a large T cell component which is capable of killing lymphoid cell lines (LCL) of B cell origin. Most LCL contain EBV and evidence has been presented which suggests that the cytotoxicity may be specific for an EBV-associated antigen. However two factors complicate the hypothesis. First, the generation of cytotoxic cells is not confined to EBV-induced disease but also occurs in mononucleosis caused by CMV; second, even in EBV-IM there is cytotoxicity for EBV-negative LCL which is associated with atypical blast cells.

We propose to examine the conditions and kinetics of induction of atypical cells in vitro by EBV antigens or by EBV in association with the B cell. The functional capacities of the cells will be assessed with particular reference to their cytotoxic and regulatory functions and will be compared with those of similar cells induced in vitro by CMV antigens or in vivo in patients with IM.

The ultimate objectives of the proposal are to determine whether the atypical cell is a common link between IM induced by various agents, why it is particularly prone to induction by EBV and whether this may have general implication for the control of proliferative disease.

64. AUTOLOGOUS LYMPHOCYTE REACTIONS AND IMMUNE SURVEILLANCE

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The capacity of lymphocytes from acute leukemic patients in remission to generate an autologous mixed lymphocyte reaction and to generate allospecific cytotoxic T lymphocytes will be determined. These preliminary studies will form the basis of our attempts to generate anti-leukemic cytotoxic T cells. Cryopreserved leukemic blasts will be added to remission T and B cells in an autologous mixed lymphocyte reaction. Finally, anti-leukemic cytotoxic T cells will be grown up in continuous culture using T cell growth factor.

BIBLIOGRAPHIC REFERENCES: Kuntz, M.M., Innes, J.B., and Weksler, M.E. The cellular basis of the impaired autologous mixed lymphocyte reaction in patients with systemic lupus erythematosus. J. Clin. Invest., 63:151, 1979. Goidl, E.A., Cusana, A., Redner, R., Innes, J.B., Weksler, M.E. and Siskind, G.W. Studies on the Control of Antibody Synthesis. XV. Effect of Nonspecific Immunodepression on Antibody Affinity. Cell Immunol. 47:293, 1979.

65. GENERATION OF CYTOTOXIC LYMPHOCYTES AGAINST LYMPHORETICULAR MALIGNANCIES

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The objective of the proposed research is to generate large numbers of cytotoxic lymphocytes in vitro, capable of lysing autologous human leukemia and lymphoma cells, that could ultimately be used immunotherapeutically in patients following tumor cytoreduction. Attempts will be made to generate cytotoxic T lymphocytes (CTLs) by culturing patients' T cells in vitro with 1) autologous malignant cells and allogeneic normal cells (in 'three-cell' protocols), 2) allogeneic malignant cells of the same type as the patient's, 3) allogeneic normal lymphocytes pooled from 20 individuals, or 4) concanavalin A. CTLs generated by these methods will be restimulated or grown in media containing T cell growth factor to generate large numbers of CTLs. These CTLs will be used for studies aimed at determining whether malignant cells from different patients share common target antigens. To determine whether in vitro generated CTLs can inhibit leukemia development or progression in vivo, mouse CTLs that lyse syngeneic leukemia cells will be generated in vitro by similar protocols and will be tested in vivo in immunoprophylaxis and immunotherapy protocols.

66. EXPLOITATION OF LONG-TERM LYMPHOCYTE CULTURES IN PATIENTS WITH CANCER

Knight, R. A., Fitzharris, P., Iliescu, V., Souhami, R., University of London, University College Hospital Medical School, Dept. of Clinical Haematology, *Chenies Mews, London WC1, England, United Kingdom*

OBJECTIVE: A comparison in man of T lymphocyte function, with particular emphasis on cytotoxic T cells in normal individuals and cancer patients.

APPROACH: 1) The detailed fractionation of fresh lymphocytes from normal donors and cancer patients, and analysis of the cytotoxic activity of the various fractions on a spectrum of cultured normal and tumor target cells. 2) The effect of short-term and long-term T lymphocyte culture, with or without tumor cell immunization in vitro, on the magnitude and specificity of cytotoxicity.

PROGRESS: 1) Spontaneous killing by fresh lymphocytes is mediated by more than one lymphocyte fraction, including cells indistinguishable from T lymphocytes. 2) Short- and long-term cultured T cells are cytotoxic for many cultured tumor cells, even after intentional and irrelevant stimulation. 3) T cells cytolytic for target cells not susceptible to lysis by fresh T cells can be induced by short-term culture with several target cell lines, and the ability to induce such cytotoxic T cells can be transferred by supernatants from the original tumor cell cocultivation. 4) T cells which augment or suppress spontaneous killing by T and non-T fractions can be induced by similar cocultivation with tumor cell lines.

67. USE OF MONOCLONAL ANTIBODIES IN STUDIES OF HUMAN CYTOTOXIC T CELL FUNCTION

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This proposal describes a series of experiments for in vitro characterization of functional human T cell subsets as defined by monoclonal antibodies. Initial description of four monoclonal antibodies that recognize human T cells has been completed. The T cell subsets will be examined for their role in generating a proliferative and cytotoxic response against allogeneic and haptenated autologous stimulator cells. These studies will use human lymphoid cells enriched for or depleted of a particular subset by fluorescence activated cell sorting and by antibody plus complement treatment respectively.

68. IN VITRO SENSITIZATION OF HUMAN LYMPHOCYTES

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Recent evidence has shown that cytotoxic T lymphocytes (CTLs) specific for surface modifications on autologous cells play a critical role in the effector mechanisms important in the destruction of tumors and in autoimmunity. Precise analysis of the cell-cell interactions, homeostatic regulation and genetic principles governing the generation of these CTLs in man has been facilitated by 1) the development of in vitro techniques for the sensitization of human lymphocytes to major histocompatibility complex (MHC) identical leukemic cells and to chemically modified (e.g., TNP-modified) autologous cells and 2) the development of methods for the isolation of functionally unique subclasses of human T cells. Thus, we have shown that human T cells can be triggered in vitro by MHC identical leukemic blasts or TNP-modified autologous cells to differentiate into CTLs which specifically lyse 51Cr labelled target cells bearing surface antigens in common with the stimulating cell. Further, this differentiation process is facilitated by helper signals provided by interaction with either allogeneic cells or soluble antigens. Our objectives in this grant are to 1) investigate in detail the role of human T cell subclass interactions in the induction and homeostatic control of the differentiation of CTLs which specifically recognize altered surface determinants on autologous cells and determine whether more than one T cell subclass is required and whether suppressor or helper cells modulate this response; 2) study the mechanisms by which regulatory signals facilitate the induction of altered-self reactive CTLs; 3) investigate the nature of target cell surface antigens recognized by MHC identical CTLs, and determine whether these killer cells recognize, at least in part, target cell MHC determinants; and 4) extend these studies to the analysis of T cell mediated altered-self cytolysis in patients with leukemia and in patients with autoimmune diseases.

69. HUMAN CYTOTOXIC CELLS

Knight, R. A., Fitzharris, P., Iliescu, V., Imperial Cancer Research Fund, Tumor Immunology Unit, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

Fresh unstimulated lymphoid cells from normal donors as well as from cancer patients kill a variety of long-term cultured tumor cell lines. We have shown that this 'spontaneous killing' (SK) is mediated in normal individuals by both T and non-T cytotoxic effector cells on four cultured tumor target cells. A fifth tumor target (MDA-157) derived from a breast carcinoma, however, is not killed by fresh T cells. We have, accordingly, asked whether fresh T cells from patients with breast cancer are cytotoxic for MDA-157, but, currently, have failed to find evidence of tumor-related T cell sensitization.

Although fresh T cells do not kill MDA-157, T cell cytotoxicity of this target can be induced by culture with some, but not all, tumor cell lines. In particular, culture of T cells with MDA-157 itself does not result in the generation of cytoxocity for MDA-157 target cells. If, however, a mixture of T and null cells is stimulated with MDA-157, killing activity on this target is generated. Preliminary experiments suggest that this induction of cytotoxicity in the mixed responder population depends on an interaction between T and null cells.

A similar interaction between T and null cells has been demonstrated at the cytotoxic effector stage. T cells cultured with interferon-containing supernatants from a previous mixed lymphocyte-tumor cell culture, although not themselves cytolytic, augment cytotoxicity by fresh null cells. This cellular interaction is not restricted at any HLA locus.

70. CULTURED TUMOR-SPECIFIC CYTOTOXIC LYMPHOCYTES

Smith, K. A., Dartmouth College, Dartmouth Medical School, Dept. of Medicine, *P.O. Box 833, Hanover, New Hampshire*, 03755, United States of America

OBJECTIVES: Demonstrate the tumor-antigen reactivity of human and murine cytotoxic T-cell lines. Test in vivo the immunoprophylactic and immunotherapeutic value of murine cytotoxic T-cells maintained in continuous proliferative culture. Characterize and purify the growth factor which allows for the long-term culture of cytotoxic lymphocytes.

71. STUDIES OF TUMOR IMMUNITY WITH MONOCLONAL T LYMPHOCYTES

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The objectives of our research are to develop monoclonal lines of T lymphocytes which are cytotoxic to syngeneic tumor targets, characterize the T lymphocyte cell lines, and use these cytotoxic T lymphocytes to study mechanisms and regulation of cell-mediated immunity to tumor cells. Previously, in vitro systems used in studying cellular immunity to tumors have employed heterogeneous populations of tumor immune cells. Recently, however, methods have been developed for cloning immune T-cells and maintaining these cells indefinitely in culture (Gillis, S., and Smith, K.A., 1977, Nature 268, 154; Nabholz et al. 1978, Current Topics in Microbiology and Immunology 81: 176). Using this new methodology we intend to develop a monoclonal population of immune killer T-lymphocytes and to characterize the interaction of these killer T-cells with the tumor target cell line. We also intend to characterize suppression of this system, which is probably mediated by soluble suppressor factors produced by suppressor T-lymphocytes.

Our strategy for undertaking this work is, first, to establish and characteize clones of mouse spleen anti-tumor T-cells; second, to define the interaction of the cloned immune cells with the tumor target cell line; third, to investigate suppression of the well defined killer-target cell systems by soluble suppressor factors; and fourth, to characterize biochemically the T-cell receptors and suppressor factors of this system.

72. ACTIVATION AND PROPERTIES OF CYTOTOXIC T LYMPHOCYTES

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OBJECTIVE: To investigate the nature of the cellular interactions required for the activation and regulation of cytotoxic T lymphocytes.

APPROACH: We have established that the minimum conditions required for the activation of cytotoxic precursors are alloantigens and co-stimulator, a lymphokine obtained by stimulation of spleen cells with concanavalin A. Using this culture system one can accurately estimate the number of cytotoxic precursors reactive to alloantigens, TNP-modified self antigens or syngeneic tumors. Furthermore, about 1 in 30 cultures containing single lymphocyte, co-stimulator and concanavalin A gave rise to single cytotoxic clones after a 5-day incubation period, strongly suggesting that co-stimulator can act directly on alloantigen or mitogen-activated cells. This extremely sensitive culture system will be used to provide unequivocal data regarding the Lyt phenotype of cytotoxic precursors to alloantigens, TNP-modified self antigens, or syngeneic tumors. The nature of the cellular interactions underlying co-stimulator production in a mixed lymphocyte reaction will also be investigated.

73. CHARACTERIZATION OF LYMPHOID POPULATIONS IN CANCER

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In this project we wish to define parameters which determine the capacity of lymphoid cells and tumor cells to serve as stimulators in the mixed lymphocyte reaction and for the generation of cytotoxic T cells. The turnover and shedding rates of membrane alloantigens are being analyzed at the molecular level, and cellular processes controlling metabolism of these antigens are being studied. Factors produced by cytotoxic T cells which suppress specifically the immune performance of sensitized T cells are being isolated and characterized.

74. CELL SURFACE EVENTS IN CELLULAR IMMUNITY

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Thymus-derived lymphocytes (T cells) serve as effectors of cell-mediated immunity to allografts and to syngeneic virus-infected cells and tumor cells. These activities rely on subpopulations of T cells called cytotoxic T lymphocytes (CTLs) and evidently require specific and direct contact between CTL and target cell. It is the aim of this proposed research to study the cell surface phenomena (i.e., membrane macromolecule interactions) that give rise to specific T cell-mediated immune reactions.

The experimental approach will utilize several model systems in vitro which mimic physiological T cell-target cell interactions. These include: (1) a transplantable mouse plasmacytoma, MOPC-315; (2) mouse fibroblasts infected with vesicular stomatitis virus (VSV); (3) chemically modified mouse spleen cells; and, (4) membrane fragments derived from the above cellular systems. The following investigations are planned: (1) the binding avidity of CTLs for their specific targets under various conditions; (2) the requirement of antigen aggregation on target cell surfaces for effective CTL activity and the influence of membrane fluidity on this activity; (3) the effects of plant lectins (such as concanavalin A) on CTL-target cell interactions; and, (4) the use of spectroscopic probes (e.g., fluorescence and electron spin resonance) to examine the interactions of cell surface macromolecules involved in these events.

75. THE ROLE OF THE T145 GLYCOPROTEIN IN THE FUNCTION OF CYTOLYTIC T-LYMPHOCYTES

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OBJECTIVE: We want to determine if T145, a surface glycoprotein which is expressed specifically on mature cytolytic T cells, is participating in the molecular events that underlie the cytolytic activity of these lymphocytes.

APPROACH: The approach is based on somatic cell genetics and biochemical analysis of established cell lines expressing the CTL phenotype.

PROGRESS: The glycoprotein T145 has been identified on several CTL-lines and has been purified from mitogen activated T cells.

76. BIOCHEMICAL STUDY OF THE CYTOLYTIC T LYMPHOCYTE

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The main objective of this proposed research is the biochemical resolution of cytolytic T lymphocyte (CTL) mediated foreign cell lysis.

An antigen from allogeneic tumor cell membranes, believed to be part of the major histocompatibility complex, has been found capable of stimulating a CTL response in vitro. One part of this project is to use this antigen to characterize the CTL receptor. The antigen will be radioactively labelled with iodine and the binding properties of the CTL receptor for antigen determined. If the antigen-CTL receptor interaction is specific and of high affinity, the labelled antigen will be employed to isolate the CTL receptor.

The process of CTL lysis of foreign cells will be investigated to determine the biochemical events leading up to foreign cell lysis. One objective is to determine if the foreign cell is directly lysed by the CTL or if it is first killed, with lysis a secondary event. To accomplish this a very sensitive assay for foreign cell lysis will be developed based on enzyme release. The marker for cell death will be the ATP level of the foreign cell.

77. CELLULAR INTERACTIONS REGULATING THE CYTOLYTIC T LYMPHOCYTE RESPONSE

Burakoff, S. J., Sidney Farber Cancer Center Inc., Dept. of Pathology, *44 Binney St., Boston, Massachusetts,* 02115, United States of America

The general aim of my research is to develop the capacity to regulate or manipulate the cytolytic T lymphocyte (CTL) response; especially, the CTL response to viral infections and malignancies. It has become evident that the cellular and humoral immune responses involve a complex series of regulatory T cell interactions. Though the evidence for helper and suppressor T cells that modulate the humoral response has existed for several years, a role for these cells in regulating the CTL response is only just beginning to surface. Therefore, in order to accomplish my more general goal, my specific aims are directed at defining the complex cellular interactions that regulate the CTL response.

The specific aims of this proposal, therefore, are as follows: (a) To define the role of the suppressor T cell in the CTL response. Specifically, the suppressor pathways that regulate the CTL response to trinitrophenyl (TNP) modified syngeneic cells in vivo will be defined. (b) To define the role of the helper T cell in the CTL response. Attempts will be made at enriching for helper T cells and at establishing in vitro lines of helper T cells. (c) To define the role of adherent cells and/or macrophages in the CTL response. It will be determined whether adherent cells present antigen to the T helper cell or pre-CTL.

Thus, the major cell interactions that regulate the humoral response will be investigated in the CTL response. Initially these parameters will be established in the CTL response to TNP modified syngeneic cells. This is a well-defined and easily manipulated system with which I have a great deal of experience. Once these cellular interactions are delineated for the TNP system, these cellular interactions will be investigated in the CTL response to Sendai virus and influenza virus.

78. INTERACTION OF CYTOTOXIC T-LYMPHOCYTES WITH MODEL MEMBRANES

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A model membrane bilayer experiment will be designed to discover 'recognition rules' which determine under what conditions cytotoxic T lymphocytes recognize targets. Histocompatibility proteins and various membrane antigens will be incorporated into giant (100 micron) lipid vesicles (spherical membrane bilayers). The giant vesicles will be converted into a horizontal planar membrane separating aqueous chambers, suitable for electrical measurements similar to the black lipid membrane experimental system. Electrical conductance will be measured as cytotoxic T lymphocytes are allowed to settle on the bilayer. This project will attempt to discover patterns of time-dependent electrical conductance increase across the membrane which correlate with attack by cytotoxic cells which is histocompatibility type specific. The synthetic antigen, stearoyl TNP dextran, will be incorporated into the model membrane bilayer along with histocompatibility protein from the K region of the H-2 locus. Cytotoxic T lymphocytes stimulated by trinitrophenol (TNP) modified syngeneic cells will be allowed to settle on the model membrane, and electrical conductance will be measured. This result will be compared with the case in which TNP-modified histocompatibility protein is incorporated into the model membrane bilayer, as a test of the 'dual recognition' hypothesis.

79. STUDIES ON ONTOGENY AND SPECIFICITY OF VIRUS-SPECIFIC CYTOTOXIC T CELLS

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The summary for this project is under entry no. 13.

80. RECOGNITION AND KILLING BY CYTOTOXIC T LYMPHOCYTES

Engelhard, W. H., University of Virginia, School of Medicine, Dept. of Microbiology, *Box 246, Charlottesville, Virginia*, 22901, United States of America

The proposal seeks to understand the mechanisms by which cytotoxic T lymphocytes (CTL) recognize and kill target cells. The work is based upon the availability of large quantities of purified human histocompatibility antigens and an in vitro system in which mouse CTL precursors are induced to differentiate upon being presented with the antigen reconstituted into phospholipid vesicles. This induction is antigen-specific, and the CTL formed specifically recognize the appropriate antigens on the target cell surface. A binding assay will be developed in which CTL interact with radiolabelled histocompatibility antigens in phospholipid vesicles. This will allow characterization of the binding. Antigen-containing vesicles will also be used in an attempt to inhibit the target cell lysis. The proposal also will examine the cytotoxic mechanism by using histocompatibility antigen-containing vesicles or erythrocyte ghosts to which these vesicles have been fused, as model targets in order to assess the influence of target cell membrane composition and organization on susceptibility to lysis. In addition, by the use of internal markers of various sizes, the size of the initial lesion will be determined. The availability of the present system, and the considerable precedent for such an approach, suggest the feasibility of using immobilized histocompatibility antigens as immunoadsorbents for the purification of antigen binding molecules from T cells (receptors). By using biosynthetically labelled T cells, attempts will be made to purify and characterize such molecules. Finally, the production of antisera specific for differentiated CTL and the use of cell monolayers as adsorbents are contemplated as a means for the isolation of molecules unique to differentiated lymphocytes.

81. REGULATORY SIGNALS IN CYTOTOXIC T-CELL DEVELOPMENT

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The objective of this proposal is to delineate the precise role that IgG antibodies, surface antigens and antigen-antibody complexes derived from surface antigens and their respective antibodies play in regulating cytotoxic T-cell development. The primary focus of this investigation will be on demonstrating that a primary cytotoxic T-cell response is regulated by an antigen-driven suppressor cell whereas a secondary response is modulated by an antigen-antibody complex-triggered suppressor cell. To induce suppressor cells in primary immune response, heat-inactivated tumor cells will be used. Secondary cytotoxic T-cell response can be induced by heat-inactivated cells and inhibited by antibodies. The observed inhibition by antibody-coated cells appears to be mediated by antigen-antibody complexes.

We are studying the effects of antigen vs. antigen-antibody induced suppression in a well-defined in vitro induction system, using 51Cr-release assay for cytotoxic activity of T cells.

These studies will allow characterization of the suppressor cells involved with respect to drug sensitivities, surface markers and adherence properties.

82. RECONSTITUTION OF TARGET VESICLES FOR SPECIFIC T-LYMPHOCYTE MEDIATED LYSIS

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We have proposed a scheme to reconstitute lipid vesicles containing plasma membrane proteins that can be recognized and lysed by thymus derived lymphocytes. Such reconstituted vesicles could then be used as a model system for further investigation of the involvement of the membrane in T-cell recognition, programming and subsequent cytolysis.

The properties of an existing although unsatisfactory reconstituted target vesicle system suggest that a high protein:lipid ratio will be required for vesicles to be susceptible to lysis. Since proteins in erythrocyte membranes are relatively immobile our first approach is to incorporate solubilized erythrocyte membrane proteins into vesicles. The second planned approach is to incorporate large amounts of membrane associated actin along with the major histocompatibility antigen, H-2, using the exfoliates of P815 tumor cells which have been shown to be highly enriched in these proteins.

83. MOLECULAR BASIS FOR T-CELL KILLING OF TUMOR CELLS

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The overall aim of this proposal is to extend our understanding of the mechanism of tumor cell killing by cytolytic thymus-derived lymphocytes (CTL). We would like to use specific antibody directed against T cell membrane components in order to identify CTL molecules which may be involved in the lytic mechanism. These antibodies are being synthesized using the recently available technique of obtaining permanent hybrid cell lines which secrete antibodies (hybridomas).

In a second, complementary approach to the mechanism of tumor cell lysis by CTL, we would like to determine if TSL is a membrane attack mechanism, and if so, what are the earliest changes in the tumor cell with regard to its permeability properties and effect on ion fluxes and membrane potential.

84. THE IN VITRO AND IN VIVO ANTIGENIC REACTIVITY OF LONG-TERM TUMOR-SPECIFIC CYTOTOXIC T-CELL LINES

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Although mixed tumor cell-lymphocyte cultures (MTLC) have been successful in generating tumor-specific cytotoxic T lymphocytes in highly antigenic, virus-producing, experimental tumor systems, similar approaches have been unsuccessful where tumor regression has not been demonstrable in vivo. Because the generation of cytotoxic lymphocytes in vitro has been shown to enhance in vivo tumor rejection upon adoptive transfer, this technique may offer unique methods for immunotherapy. For this technique to be of practical value, however, it will be necessary to generate lymphocytes cytotoxic to weakly antigenic tumors and to cultivate these cells in large quantities.

We have been successful in the generation of murine tumor-specific cytotoxic lymphocytes which lyse weakly antigenic nonvirus-producing (nonproducer) tumor cells by using two methods: 1) repetitive MTLC of normal murine spleen cells with allogeneic nonproducer leukemia cells and 2) repetitive MTLC of producer-immune spleen cells with syngeneic, highly antigenic, virus-producing leukemia cells. Evidence from these studies suggests that the antigenic specificity of these cytotoxic lymphocytes may be directed against sarcoma and/or transplantation-specific antigens. Demonstrations of anti-tumor immune reactivity against virus replication-defective murine leukemia and sarcoma cells are particularly significant due to the animal model system's similarity to the human leukemic condition.

In addition, we have been successful in the long-term exponential in vitro cultivation of differentiated, tumor-specific cytotoxic T-lymphocytes. This has been made possible through the use of a T-cell growth factor present in medium conditioned by mitogen-stimulated normal lymphocytes.

The objectives of this research proposal are to generate long-term cytotoxic T-cell lines with reactivities directed against sarcoma and/or transformation-specific antigens expressed by replication-defective murine leukemia and sarcoma cells, to explore the efficacy of these cells in in vivo tumor rejection and protection and to characterize, concentrate and purify the growth-promoting factor(s) which allows for their proliferative in vitro culture. Additionally, we will attempt to enhance the in vitro generation of tumor-specific cytotoxic T-cells through the addition of T-cell growth factor to MTLC. (Text Abridged.)

85. THE ROLE OF THE H-2L LOCUS ANTIGENS IN T-CELL MEDIATED CYTOTOXICITY

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BALB/c H-2db spleen cells generated a strong primary in vitro CML response against BALB/c (H-2)d wild type spleen cells. The effector cells generated were specific for L antigens. In an allogeneic CML response between two non-mutant strains directed against H-2D region antigens, two clones of cytotoxic effector cells could be identified, one with specificity for D antigens, and the second with specificity directed against L antigens. When syngeneic cytotoxic effector cells were generated against TNP modified H-2D antigens, no clones with specificity for L-TNP modified antigens could be detected. This contrasts with the H-2 restricted murine influenza model, in which influenza virus can be recognized by cytotoxic T cells in association with products of the L locus. Therefore, although products of the L locus are capable of inducing allogeneic cytotoxic effector cells, and serve as self recognition structures for influenza virus, these antigens appear not to be capable of eliciting syngeneic effector cells specific for the TNP hapten.

BIBLIOGRAPHIC REFERENCES: Levy, R. B. and Hansen, T. H.: Functional studies of the products of the H-2L locus. Immunogenetics 20: 7, 1980.

86. ELICITATION OF CYTOLYTIC EFFECTOR CELLS WITH SOLUBILIZED ANTIGENS

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We propose to study the mechanism by which an IgA myeloma tumor, MOPC-315-EL (BALB/c origin), undergoes a periodic loss, during each transplantation cycle, of the ability to interact with thymus-derived lymphocytes (T). Our approaches will center around the evaluation of the T lymphocyte-tumor cell interaction and the structural change (s) which is responsible for the altered reactivity. The cellular interactions between T cells and the reactive and unreactive MOPC-315-EL tumor cells will be evaluated by 1) T lymphocyte-tumor cell conjugate formation, 2) inhibition of T cell-tumor cell conjugate formation with solubilized, enriched H-2 antigen containing fractions from the reactive and unreactive tumor cells to elicit specific cytotoxic T lymphocytes (CTLs) in culture. Structural changes which might be responsible for the reduced T cell reactivity will be studied by examination of the surface protein, lipid, and carbohydrate composition of the reactive and unreactive MOPC-315-EL tumor cells. A study of the in vivo relationship between the host (BALB/c mouse) and the tumor will be carried out by establishing 1) whether there exists heterogeneity of the MOPC-315-EL tumor cell population, 2) whether a soluble factor is responsible for the induction of the change in reactivity to T cells, and 3) whether this change in reactivity to T cells is an important tumor escape mechanism.

87. MEMBRANE ANTIGEN ORGANIZATION IN TUMOR IMMUNITY

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The lymphocytes from animals immune to syngeneic simian virus 40 (SV40)-induced tumors recognize both virus-induced and histocompatibility-2 (H-2) antigens on SV40 transformed target cells. Evidence presented here suggests that T cells bind to a complex between H-2 and the viral antigen on the tumor cell, and formation of this complex is necessary, if not sufficient, for induction of cytolytic T cells and for in vitro tumor destruction by T cells. In the work proposed, we will investigate the formation of antigen complexes in the tumor cell plasma membrane and the relationship between complex formation and immune T cell recognition.

Co-capping of tumor-associated antigens with mouse H-2 antigens, reported by this and other laboratories, also indicates physical associations among plasma membrane molecules. We have recently found that antigens which co-cap can also be extracted from the membrane as specific complexes. Both the murine leukemia virus gp71 and a carcinofetal antigen, Ag I, are associated with the H-2 antigens following solubilization of L cell membranes with low concentrations of non-ionic detergents. We will study the requirements for formation of these complexes and the relationship between complex formation and endogenous virus production.

We will establish a model system for T cell recognition of foreign proteins expressed on syngeneic cells. This model will allow quantitative evaluation of immune recognition and manipulation of the immune response to cell surface antigens.

88. AUTOSENSITIZATION IN VITRO DETECTED BY CELL-MEDIATED CYTOTOXICITY

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T-cell-mediated cytotoxicity has been generated by culturing immune spleen cells from untreated donors without the addition of any known cellular antigens. The effectors generated preferentially lysed H-2 matched targets, but also lysed to some extent allogeneic target cells. High (H-2d) and low (H-2k,b) responder strains were identified, and strong responsiveness was a dominant characteristic. Specificity studies indicated that multiple clones of cytotoxic cells were generated in these cultures. Separate clones of effector cells could be identified which were responsible for the H-2 restricted and the non-restricted components. The findings suggest that the self-restricted component may be directed in part at either components present in a specific serum (i.e. fetal calf but not horse) or antigens induced by that serum.

BIBLIOGRAPHIC REFERENCES: Levy, R. B., Shearer, G. M., Kim, K. J., and Asofsky, R. M. Xenogeneic serum-induced murine cytotoxic cells: I. The generation of effector components specific for self and allogeneic target cells. Cell Immunol. 48: 276-287, 1979.

89. IN VITRO SELECTION OF LYMPHOCYTES WITH SPECIFIC ANTITUMOR CYTOTOXIC ACTIVITY

Colnaghi, M. I., DellaPorta, G., Menard, S., Istituto Nazionale per lo Studio e la Cura dei Tumori, Division of Experimental Oncology A, *1 Via G Venezian, 20133 Milan, Italy*

OBJECTIVE: To verify whether the supernatant from Con A-treated murine spleen cell cultures which induces a continuous growth of murine lymphocytes from an unidirectional mixed cell culture and selects progressively T cells specifically cytotoxic for the stimulating cells has a species specific activity. The selection of a lymphoid cell population specifically directed against a certain antigenic specificity would allow immunotherapy experiments as well as the characterization of tumor specific antigens.

APPROACH: The supernatant from Con A-treated murine spleen cell cultures will be added to human lymphoid cells recovered from various types of mixed cultures and the possible establishment of a continuous line with a specific cytotoxic reactivity will be verified.

90. ANALYSIS OF MECHANISMS OF HUMORAL AND CELL-MEDIATED IMMUNITY TO ALLOGRAFTS AND TUMORS

Brunner, K. T., Cerottini, J., Engers, H. D., Inst. Suisse de Recherches Experimentales sur le Cancer, Dept. of Immunology, *Chemin des Boveresses, CH1066 Epalinges, Switzerland*

OBJECTIVE: Study of the mechanism of in vitro induction and of the in vitro and in vivo functional activity of cytolytic T lymphocytes (CTL) in allograft and MSV induced tumor immunity in the mouse.

APPROACH: Generation of CTL in mixed leukocyte and in mixed leukocyte-tumor cell culture. Use of cell surface markers, antigen binding and cell separation techniques, and of functional assays in the characterization of responding and accessory cell types. Study of the role of nonspecific factors in CTL generation. Study of clonally expanded CTL populations and of T-cell hybridomas. Study of the specificity of individual responding T cells in limiting dilution assay.

PROGRESS: Highly cytolytic T lymphocytes were generated in vitro by repeated stimulation with allogeneic lymphocytes or with MSV induced lymphoma cells. Clonal expansion of CTL was achieved by further stimulation with nonspecific factors generated in secondary MLC. A limiting dilution assay was developed, allowing the determination of frequency and specificity of individual alloreactive and MSV tumor reactive CTL precursors.

91. ANALYSIS OF DIFFERENTIATED FUNCTIONS OF LYMPHOCYTES BY MEANS OF SOMATIC CELL GENETICS

Nabholz, M., Haas, W., Johnson, J., MacDonald, H. R., Inst. Suisse de Recherches Experimentales sur le Cancer, Genetics Unit, *CH1066 Epalinges, Switzerland*

OBJECTIVE: Identification of the genes and gene products controlling the expression of the cytolytic T cell (CTL) phenotype (specificity, cytolytic capacity, T cell growth factor (TCGF) dependence).

APPROACH: Our approach is based on a somatic cell genetic and biochemical analysis of established cell lines expressing the CTL-phenotype.

PROGRESS: We have established and characterized a number of murine CTL-lines with different genetic backgrounds and specificity. Stable drug and lectin resistant mutants have been derived from several lines. We have also shown that cytolytic T cell hybrids which can be produced by crossing CTL-lines with inactive lymphoma derived lines of the hybrids are selected and maintained in the presence of TCGF.

92. CHOLESTEROL IN NORMAL AND MALIGNANT LYMPHOCYTES

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Membrane-bound cholesterol in lymphocytes can be manipulated by treating these cells with oxygenated derivatives of cholesterol, which specifically depress the de novo synthesis of cholesterol. Inhibition of cholesterol synthesis in lymphocytes abolishes their DNA synthesis and thus interferes with cell division. In addition, specific immunological functions of T-lymphocytes, such as target cell killing, are abolished. We propose to analyze the mechanisms through which membrane-bound cholesterol is involved in T-cell killing. The importance of de novo synthesis of cholesterol for the induction of a secondary, cell mediated response will be analyzed in detail. These studies will be concerned particularly with the sterol and phospholipid composition of the plasma membrane of immunocompetent cells. Some sera used for tissue culture purposes are inhibitory to lymphocytes and prevent proliferation either induced by lectins or by an allogenic lymphocyte reaction. We propose to investigate whether the inhibitory activity of such sera resides in the lipids, specifically in sterols of the lipoprotein fraction.

The role of membrane bound cholesterol for antibody production and secretion will be investigated in MOPC-104E plasmocytoma cells as well as in B-lymphocytes stimulated by lipopolysaccharide (LPS). A series of experiments is also directly concerned with the structure of the LPS receptor on B-cells and its dependence on the lipid composition of the plasma membrane.

The regulation of the synthesis of cholesterol in lymphocytes and leukemic cells will be investigated by studying the structural requirements of the inhibitory sterol molecules and by examining the binding of non-inhibitory sterols to cytoplasmic receptors in normal and malignant lymphocytes. The effects of oxygenated sterols such as 25-hydroxycholesterol or 7-ketocholesterol, administered to animals in vivo, through the diet or intraperitoneally, will be analyzed. We will especially focus on the effects of such treatments on the cell mediated and humoral immune response in the intact animal as well as on their effects upon the growth of transplanted and spontaneous tumors.

93. T-CELL MEDIATED SUPPRESSION OF TUMOR GROWTH

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Previous studies from our laboratory have shown that rat lymphoid cells which were stimulated in an in vitro secondary immune response to a syngeneic virus-induced lymphoma developed the capacity to kill lymphoma cells and to inhibit tumor growth in vivo. The demonstration that the induction of cytotoxic T-lymphocytes (CTLs) involved the participation of tumor virus-specific helper T-cells responsive to antigens of murine leukemia virus (MuLV) represented a novel finding of these studies.

In the present application we propose to continue studies in inbred mice to examine the nature of CTLs and CTL precursors which mediate inhibition of the growth of MuLV-induced syngeneic tumors and the availability of effector cell precursors in mice with progressively growing tumors. Additional experiments will explore the role of purified helper and suppressor T-cells and macrophages in regulating the induction of antitumor CTL responses in vitro. The role of each cell type will be first defined in normal and/or immune mice and then examined in mice with progressively growing tumors. The influence of purified viral antigens shed by tumors on the induction of effector cell responses will be further defined. These studies should clarify our understanding of host cellular immune mechanisms which regulate antitumor effector responses; they may provide a rational basis for inducing antitumor effector activity by otherwise unresponsive cells from mice with growing tumors.

94. STUDIES ON CYTOTOXIC T LYMPHOCYTES

Miller, R. G., Ontario Cancer Inst., Division of Biological Research, *500 Sherbourne St., Toronto, Ontario*, M4X 1K9, Canada

OBJECTIVES: To elucidate the mechanisms involved in the production of both cytotoxic T lymphocytes (CL) and their precursors (CLP).

APPROACH: Using quantitative in vitro culture techniques and inbred mice, one can measure relative CL number, CLP frequency, CL per CLP, helper cell activity and suppressor cell activity. Using this underlying technology, two particular approaches are planned: 1) Using a flow cytometer-cell sorter and the fluorescent vital stain H33342, one can detect and sort specific CLP within 12 hrs of culture initiation. This enables one to decouple the two signals required for CLP activation and should enable a more complete dissection of the cellular requirements for CL production than previously possible. 2) T cell colonies can be grown from normal mouse bone marrow and from the spleens of normal and athymic nude mice. The colonies from each tissue contain a variety of functional cell types (CLP, helper, suppressor) characteristic of the tissue. The Lyt and Ia antigen distributions within individual colonies will be measured by flow cytometry and correlated with function. Colonies containing CLP contain CLP of several specificities and it will be investigated whether diversification is occurring within the colony.

95. T-LYMPHOCYTE-MEDIATED CYTOLYSIS

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This is part of a broader project. A summary of this subproject is not available.

4. NON-SPECIFIC AND OTHER T CELLS

96. DISTRIBUTION AND FUNCTION OF LYMPHOID SUBPOPULATIONS IN ACUTE LYMPHOBLASTIC LEUKEMIAS AND LYMPHOMAS

Sen, L., Estevez, M. E., Finiasz, M., Diez, R., Guintoli, J., Instituto de Investigaciones Hematologicas, Depto. de Microbiologia, *Pacheco de Melo 3081, 1425 Buenos Aires, Argentina*

OBJECTIVES: To study the relationship between alterations in immunocompetent cells and in the development or progress of leukemias and lymphomas. To determine in untreated patients with ALL or lymphomas if the presence of blasts that express different markers is related to initial clinical parameters and response to treatment. To identify the area of cellular immune system affected by chemotherapy and determine if the immunosuppressive and immunomodulatory effects of treatment depend upon treatment duration. To determine the existence of tumor-specific antigens in lymphoblasts in ALL and lymphomas. To determine whether the reactivation of the immune system that follows the discontinuation of all chemotherapy in ALL patients is related to the individual clinical course.

METHOD: Studies will be performed at various stages of disease. All patients come from the Argentine Group for Treatment of Acute Leukemia (GATLA). Stage I: The characteristics of blast membrane in ALL and lymphomas prior to treatment will be studied. Stage II: During continuous and complete remission functional studies of the specific and non-specific immune system will be performed. Stage III. After three years of treatment and maintenance the patient in continuous and complete remission, upon suspension of treatment, will undergo studies on the distribution of lymphoid sub-populations and functional studies of the T and B systems and mononuclear phagocyte function.

PROGRESS: Our preliminary studies show that, in 58 patients studied, 9 were ALL-T; 42 were ALL null; 6 were ALL with C3; and 1 was ALL with receptors for rat red blood cells. Patients were followed for 3 to 28 months. In the ALL-T group 78% relapsed. In the ALL null group 10% relapsed. In the ALL C3 group 50% relapsed. At 19 months the patient with receptors for rat red blood cells is in complete and continuing remission. (Translated)

97. SUBPOPULATIONS OF LYMPHOCYTES IN CHRONIC LYMPHOCYTIC LEUKEMIA

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OBJECTIVE: Our objective in studying patients with chronic lymphocytic leukemia (CLL) was to correlate deterioration in clinical state with T cell function. The objective was broadened to include the determination of the natural history in terms of change of lymphocyte subpopulation and elucidation of the basis of abnormal function of affected cells.

APPROACH: T and B lymphocyte markers were identified by markers such as E rosetting, surface immunoglobulin and EAC rosettes and T cells were separated by E rosetting. Functional responsiveness of unseparated mononuclear cells and separated T lymphocytes was assessed by stimulation with phytohemagglutinin (PHA), with allogeneic lymphocytes in mixed lymphocyte culture (MLC), and with autologous leukemic B cells in the autologous mixed lymphocyte reaction (AMLR). In vitro suppressor cell function of mononuclear populations in chronic lymphocytic leukemia was studied by the addition of indomethacin, co-culture of CLL cells with normal cells and in vitro aging of mononuclear populations from CLL.

PROGRESS: Results to date suggest the following: 1) T cell responses to mitogens and to allogeneic lymphocytes are depressed. 2) Auto-stimulation of T cells by leukemic B cells is associated with the change in clinical status of disease. 3) As disease progresses there is temporal increase in T cell numbers. 4) We have not been able to demonstrate suppressor T cells.

98. LYMPHOCYTE MARKERS IN LYMPHATIC LEUKEMIA - POSSIBLE CLINICAL IMPLICATIONS AND STUDY ON SUBPOPULATION OF T LYMPHOCYTES

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On the assumption that at least some differentiation antigens for human subsets of T lymphocytes are polymorphic, we have developed an immunization program in order to avoid production of antibodies against other HLA control structures which could mask the presence of antibodies against these subpopulations.

Through a planned immunization program involving HLA-A and B compatible donors, antisera recognizing subsets of human T lymphocytes can be produced. In collaboration with Dr. Lorenzo Moretta and Marco Colombatti from Istituto di Microbiologia, University of Genova (Italia), we have studied the correlation between cell subsets as defined by antisera and T cell subpopulations identified by surface Fc receptors either for G or M immunoglobulins (T mu and T gamma).

The preliminary conclusions are as follows: Certain alloantisera obtained by planned immunization specifically recognize cells belonging to the T gamma or the T mu subpopulation; a further subdivision of the T gamma and T mu subpopulations can be done based on reactivity with some of these antisera; some antisera seem to recognize polymorphic structures controlled by HLA-linked genes.

99. PROTECTIVE T-CELL SUBPOPULATION RESPONSES IN LEUKEMIA

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This study will examine the responses of T-lymphocyte subpopulations individually and in defined combinations in vitro using four different assay procedures to detect responses to tumor cell antigens. The same populations will also be tested in vivo by a passive transfer assay to determine their capacity to protect recipients from leukemic death. The study seeks to define in vitro assays that correlate with in vivo protection.

100. BINDING OF BACTERIA TO NORMAL AND LEUKEMIC LYMPHOCYTES

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The objective of this investigation is to establish the degree of diversity of human lymphocyte subpopulations by using bacteria as markers of these subpopulations. We have develop**d** a procedure by which the whole blood cells can be labeled with bacteria, resulting in some lymphocytes appearing surrounded by bacteria in blood smears. With this procedure, we have differentiated B cells from T cells and also two B and four T cell subpopulations. We have also found that the lymphocyte subpopulations identified by bacterium endurance are functionally different. For example, the T4 lymphocytes in our system appear to be responsible for natural killing while the T2 cells are suppressor cells for this particular function. We have also found that T1T2 cells perform specific killings of allogeneic cells and also are responsible for the mitogen response of T cells. We have investigated patients with chronic lymphocytic leukemia and found that a correlation could be established between the degree of bacterial binding to the leukemic lymphocytes and the clinical status. We now propose to study the lymphocyte subpopulations identified by bacterium adherence in patients with solid tumors. This study would be carried out in a double-blind manner. We shall determine the B/T cell ratio in the peripheral blood of these patients before and after treatment, at different stages of the disease, before and after surgery, and at different time intervals after treatment. We shall determine whether the changes in the lymphocyte subpopulations in cancer patients can be correlated with a clinical status and establish the diagnostic and prognostic value of our test. We shall also follow the fate of the patients with chronic lymphocytic leukemia that have already been investigated, in terms of changes in the status and in the binding of bacteria to lymphocytes. We shall attempt to determine the possibility of automatic computerized reading of the smears developed after bacteria have been reacted with the lymphocytes, in order to obtain a test that can be used in clinical laboratories.

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101. THE PROGNOSTIC SIGNIFICANCE OF SURFACE MARKERS IN ADULT NON-HODGKIN'S LYMPHOMA IN INDONESIA

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OBJECTIVES: 1. To investigate the surface markers of lymphoma cells in Indonesia. 2. To determine the importance of surface markers in the prognosis of malignant lymphoma in Indonesia.

APPROACH: 1. The Ann Arbor symposium is used as reference for the staging procedure in Hodgkin's as well as non-Hodgkin's lymphoma patients. 2. Patients in stages I and II are treated with mantle radiotherapy, whereas the advanced cases are treated with the MOPP protocol for Hodgkin's lymphoma and the COP protocol for non-Hodgkin's lymphoma. 3. Surface markers of T and B lymphocytes are tested on the tumor cells as well as on the peripheral blood lymphocytes in all cases. 4. The Rappaport classification is used for the histological study.

PROGRESS: 1. About 150 cases have been studied preliminarily without surface marker tests. 2. About 10 cases have been completely investigated, including surface marker tests.

102. MEMBRANE PROTEINS AS MARKERS FOR LYMPHOID TUMORS

Prumers, G. F., Seijen, H. G., Rijksuniversiteit te Groningen, Dept. of Histology, *Broerstraat 5, Groningen, Netherlands*

OBJECTIVE: There is increasing evidence that subclasses of lymphoid cells have characteristic gene products expressed at the plasma membrane and that these are usually maintained in malignant disorders. Our objective is to phenotype human lymphoid leukemias and lymphomas, on the basis of plasma membrane protein patterns, and relate them to the various normal lymphoid cell populations. These studies may also provide new insights into cellular relationships, differentiation and malignancy within the immune system.

APPROACH: Phenotyping of lymphoid cell preparations is achieved by analysis of plasma membrane proteins. Thus, after 125I (lactoperoxidase method) and 3H (borohydride method) labelling of cell surface proteins, cells were lysed by detergent. The solubilized radioactive proteins were separated by SDS PAGE and detected by autoradiography.

PROGRESS: These techniques will yield suitable results only when labelling is performed under strictly controlled conditions. We were able to characterize defined cell populations in rats. From human peripheral blood lymphocytes, we isolated T- and B-enriched populations, which were clearly distinguishable by their membrane protein patterns. Also a few cases of chronic lymphocytic leukemia, Hodgkin's and non-Hodgkin's lymhpomas and a human lymphoblastoid EBV positive cell line were investigated. In all cases the protein and glycoprotein patterns were well defined and suitable for classification of these tumors. Extension of this type of analysis to other cell populations of normal and tumor origin and correlation with known markers such as Fc-and C3-receptors and membrane Ig should yield a classification system for subsets of leukemias and lymphomas related to subpopulations of normal lymphocytes.

103. ANTIGEN AND LECTIN RECEPTORS AMONG MEMBRANE GLYCOPROTEIN AND GLYCOLIPID COMPONENTS OF T AND B CELL SUBPOPULATIONS (ABBREV)

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OBJECTIVES: 1. To determine antigenic and lectin receptor differences between lymphocyte subpopulations in normals and lymphoproliferative disease. 2. To investigate why some antigens or lectin receptors appear to reside on glycolipids in some subpopulations and on glycoproteins in other subpopulations.

APPROACH: A modified form of the immunolatex assay for cell surface antigens and receptors, allowing rapid and easy detection coupled with morphological identification, is used to demonstrate the presence of various antigens or lectin receptors on lymphocytes from normals and patients with malignant lymphoproliferative disease, as well as on isolated lymphocyte subpopulations.

T and B cell subpopulations can be isolated by established techniques and compared for presence of various antigens and lectin receptors. Alternatively, the latex label can be used in conjunction with other established labels for lymphocyte subclass type such as the mouse erythrocyte rosette test or labelling with anti-immunoglobulin antibodies.

Effect of protease and other enzyme treatment will be used to determine whether the antigen or receptor is in the glycolipid or glycoprotein fraction.

PROGRESS: Preliminary evidence suggests that T cells are deficient in the cold agglutinin antigen I whereas B cells are not. Both T and B cells possess the other cold agglutinin antigens: Pr1, Pr2 and Pr3. However, the T cell Pr2 antigen is pronase-sensitive while the majority of B cells have a pronase-resistant Pr2 antigen.

104. STUDY OF LYMPHOCYTE POPULATIONS IN CORD BLOOD AND T-CELL COLONIES

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OBJECTIVE: We have investigated the lymphocyte subpopulations in cord blood (CB) by means of immunological and enzyme markers. The functional properties of T-lymphocytes in CB and adult human blood were also examined by a new technique of colony growth in vitro.

APPROACH AND PROGRESS: The proportion of T-lymphocytes (E-rosettes) in CB was significantly lower than in adult blood. Neuraminidase treatment markedly increased the percentage of E-rosettes which still remained below normal. One-third of the T-cells appear to be activated, as suggested by the percentage of H-rosettes, higher in CB than in adult blood. The levels of the enzyme terminal transferase were marginally higher in CB; in 2 out of 41 samples it was higher than in normal bone marrow. The levels of the membrane enzymes alkaline phosphatase and phosphodiesterase were also higher in CB. Our results suggest than in CB there is a three times greater than normal proportion of 'null' lymphocytes and that some immature lymphoid cell subsets may be represented.

With a double-layer technique we have obtained reproducible T-colony formation from adult peripheral blood and bone marrow. In CB samples and in cases of chronic B- and T-cell leukemias the cells showed a reduced capacity for colony formation. The factors and cell subsets involved in this phenomenon are being investigated at the present time.

105. CHARACTERIZATON OF HUMAN T CELL SUBSETS IN NORMAL AND PATHOLOGIC CONDITIONS

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OBJECTIVES: Functional characterization of human T cell subsets as defined by surface Fc receptors for IgG or IgM. Analysis of Fc receptors on T cells at different stages of their development and consequences of allogeneic stimulation for the Fc receptor expression by responder T cells. Studies of the regulatory controls exerted by T lymphocyte subsets on the colony formation by erythroid, myeloid and lymphoid populations. Analysis of surface markers and functional analysis of neoplastic cells of T cell lineage. Monitoring of T cell subsets and of T cell functions in patients who have undergone bone marrow transplantation and are developing GvHD.

APPROACH: The techniques used for isolation of human T cell subsets are based on their differential capacity for binding IgG or IgM immune complexes. For functional analysis of T cell subsets various in vitro techniques are used, such as the T-dependent response of B lymphocytes to PWM, the capacity of stem cells or PHA-activated T lymphocytes to form colonies in semisolid agar, the response of T lymphocytes in allogeneic MLR, cytotoxic assays such as ADCC, NK, cell-mediated lympholysis.

106. REAGENTS TO CHARACTERIZE HUMAN CELL SUBPOPULATIONS

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OBJECTIVES: Develop specific heteroantisera against defined populations of human lymphoid cells stressing non-malignant populations. Specifically, prepare antisera against enriched or purified preparations of T gamma, T mu, and T null cells, against T cell preparations from immunodeficient patients, and against T cell preparations that are selected on the basis of their quantitative degrees of reactivity with several presently available polyspecific anti-T cell antisera. Further, prepare antisera against B cell populations defined by various available cell surface markers, against macrophage subpopulations, and against lymphoid subpopulations that are defined by their reaction with various disease associated anti-lymphocyte antibodies. As antisera are obtained, their reactivity on various cell populations will be determined by functional depletion studies.

107. T LYMPHOCYTES AND LYMPHOTOXIN IN TUMOR IMMUNITY

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After having demonstrated losses of plasma membrane protein in target cells under the influence of allogeneic aggressor lymphocytes and alpha lymphotoxin, we are now trying to elucidate the kinetics of this process. Furthermore, we are studying subsets of lymphocytes to clarify the human T cell subpopulation which is the producer of lymphotoxin(s). These studies have a distinct bearing on tumor immunity and cellular immunity.

108. IN VITRO STUDIES OF NORMAL AND NEOPLASTIC LYMPHOCYTES

Fu, S. M., Kunkel, H. G., Chiorazzi, N., Rockefeller University, Immunology Lab., Dept. of Immunology, *66th & York Ave., New York, New York*, 10021, United States of America

Continued efforts will be made to identify differentiation block of leukemic B cells. Various human T cell subpopulations will be identified with heteroantisera. The role of these T cell subpopulations in the induction of B cell differentiation to plasma cells will be determined. A subpopulation of B cells with different sIg isotypes will be isolated from tonsillar and peripheral blood and will be subjected to differentiation induction to determine their capability in the expression of Ig isotypes intracellularly.

BIBLIOGRAPHIC REFERENCES: Chiorazzi, N., Fu, S.M., Montazeri, G., Kunkel, H.G., Rai, K., and Gee, T. T cell helper defect in patients with chronic lymphocyte leukemia. J. Immunol. 122:1087, 1979. Hansen, J. A., Fu, S. M., Antonelli, P., Kamoun, M., Hurley, J. N., Winchester, R. J., Dupont, B., and Kunkel, H. G. B-lymphoid cell lines derived from HLA-D homozygous donors. Immunogenetics 8:51, 1979.

109. RADIATION INJURY IN SUBPOPULATIONS OF LYMPHOCYTES

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Subpopulations of lymphocytes may be defined in terms of functional, morphological and immunochemical differences. Included among the properties that distinguish thymus-derived (T) from bone marrow-derived (B) lymphocytes are the apparent presence or absence of receptor sites for specific substances and the response of the involved cell type to interaction with mitogens. These differences between T and B cells reflect discrepancies in the chemical composition and structural arrangements of the plasma membrane.

B cells may be further divided into subpopulations on the basis of surface immunoglobulin. More recently, subpopulations of T cells have been defined on the basis of function and cell surface alloantigens; helper (Ly 1-positive 2-negative 3-negative) and suppressor (Ly 1-negative 2-positive 3-positive) cells are of particular importance.

Investigations to date suggest that most, if not all, of the above subpopulations differ in their sensitivity to ionizing radiation. Thus: 1) Irradiated B cells placed in tissue culture survive less well than T cells; 2) the capacity of irradiated cells to traffic in normal fashion is inhibited at lower dose levels for B cells than T cells; 3) after whole body exposure, proportionately greater numbers of T than B cells continue to recirculate and maintain viability; 4) B cells demonstrate radiation-induced morphologic alterations at lower dose levels than do T cells as documented by transmission and scanning electron microscopy; 5) T and B cells activated to appropriate mitogens or antigens in vivo and in vitro are more resistant than their nonactivated counterparts; 6) supressor T cells appear to be more radiosensitive than helper T cells; 7) a subpopulation of cytotoxic T cells is very resistant to radiation injury.

The purpose of this project is twofold: expand the above observations on the radiosensitivity of defined populations of lymphocytes with particular attention to low dose effects; define the physical-chemical basis of these differences in radiosensitivity and thereby gain a better understanding of the pathogenesis of radiation-induced interphase death of lymphocytes.

110. REGULATION OF THE IMMUNE RESPONSE

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OBJECTIVE: Various subpopulations of T lymphocytes exist, for example, cytotoxic T cells, T cells that mediate DTH, helper T cells and suppressor T cells both for antibody and delayed-type immunity. Our intention is to investigate the relationship between these functional types of T lymphocytes and attempt to relate these findings to different states of immunity or suppression during tumor progression.

APPROACH: Model systems have been established which demonstrate the following categories of T cells: a) helper T cells for antibody, b) helper T cells for DTH, c) repressor T cells for antibody and d) suppressor T cells for DTH.

The conditions under which these different subpopulations of T lymphocytes are induced will be investigated. The relationship between the individual cell populations will also be examined using Ly antisera.

111. SUBSETS OF HELPER T CELLS IN IMMUNE REGULATION

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No summary has been provided to the Smithsonian Science Information Exchange.

112. SURFACE MEMBRANE CHANGES IN T CELLS INDUCED BY SYNGENEIC TUMOR CELLS

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OBJECTIVE: To understand the mechanism by which tumors escape elimination in the early stages of their growth.

APPROACH AND PROGRESS: Injection of a large number of tumor cells, like other strong immunogenic challenges, is followed within 6 hours by the uptake of cytophilic immunoglobulin (probably complexes) by a subpopulation of T cells. This phenomenon, known as the '6-hour T cell response' is abrogated when small tumor cell inocula (10 to the second power), or small amounts of a preparation from tumor cells, which contain tumor antigens, are injected prior to the immunogenic challenge. Abrogation of the '6-hour T cell response' results in a decrease in specific antitumor cell immunity as tested in vitro by measuring growth inhibition (cytostasis). It has also resulted in loss of the amplifying function on antibody formation against sheep erythrocytes normally detected in a T-B cell co-operative system, when T cells are used 6 hours after priming with sheep erythrocytes. It is postulated that this T cell defect may represent a mechanism by which tumor cells in the early stages of their growth interfere with inductive stages of immune response for a sufficient period of time to allow the tumor to grow beyond immune control.

113. BIOPHYSICAL PROBES OF THE LYMPOCYTE MEMBRANE

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A biophysical study is proposed on the response at the lymphocyte plasma membrane level to mitogenic substances and to specific antigen binding in normal and leukemic cells. The cell triggering phenomenon, which is central to the immune response, will be initiated by known mitogenic substances and characterized in physical terms by established biophysical probes of membrane properties. The study will then be extended to specific antigen-binding subpopulations to observe membrane responses to antigen binding and B cell-T cell interactions. In order to achieve the enrichment of cell populations which will be required, a new technique is proposed for sorting large numbers of normal and leukemic cells according to surface specificities. The method (high gradient magnetic separation) depends on providing the lymphocyte subpopulation of interest with paramagnetic (e.g. ferritin-conjugated) antigens or antibodies against surface markers. Development of the magnetic cell sorting technique will first proceed using as the paramagnetic cells erythrocytes which contain some fraction of the hemoglobin iron in a paramagnetic state (ferrihemoglobin or deoxygferrohemoglobin).

114. RECEPTOR FUNCTION IN LYMPHOCYTE DIFFERENTIATION

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Our objective is to learn how receptors on lymphocyte membranes function in regulating cellular differentiation. We want to know the function of the antigen-binding receptor, whether ligands induce interaction with other types of membrane receptors, how the consequences of antigen binding are integrated with signals received from interacting cells, and how membrane organization governs receptor function. Our approach to the problem of receptor function combines two aspects: 1) developing chemical methods to visualize receptors, identify physical association or interaction of surface antigens with other membrane components, and isolate subpopulations of B and T cells on the basis of distinguishing surface markers; and 2) correlating the physical characterization of lymphocyte subsets with their function in a well-defined and readily manipulated in vitro anti-hapten antibody response. Our methods emphasize the use of hapten-sandwich procedures (hapten-conjugated antigens, antibodies, and cell surfaces in combination with anti-hapten antibodies) to induce the selective bridging of various receptors on the same or different cells, to purify cells that bear particular receptors, and to deliver effectively biologically active molecules to selected cell populations.

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115. IMMUNOCHEMICAL PURIFICATION AND CHARACTERIZATION OF IMMUNOCYTES AND THEIR COMPONENTS

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Our goal is the development of cell separation methods for the specific isolation of immune cells, particularly for varieties of antigen-reactive cells (ARC) involved in cellular immune reactions, and for their subcellular fractionation in order to study the mechanisms involved in the development of immune reactivities and immune macromolecules. Populations of cells containing ARC from normal mice and from mice immunized with allogeneic tumor cells and normal cells are tested for binding to the cell surface antigens to target cells attached to insoluble supports. Nonadherent cells are tested for depletion of cytotoxic effector cells (CTL) by measurement of 51Cr release from target cells. Graft-versus-host (GVH) activity is measured by the Simonsen assay in neonatal mice. The mixed lymphocyte culture is used to test for stimulation of ARC by alloantigen in vitro, and for the generation of CTL from their precursors (CTLp). T cell subpopulations from thymus and spleen are prepared and characterized with specific reagents such as peanut agglutinin and antibodies to the LyT and CTL differentiation antigens. Surface molecules of target cells are isolated to test their binding to ARC.

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116. LYMPHOCYTE SUBSETS AND IMMUNE RESPONSES IN MICE

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We have continued our studies of T lymphocyte immune responses and cell surface phenotypes in the mouse, concentrating on two areas. Firstly, we have further characterized by serological and genetic criteria a panel of allo- and monoclonal sera which define various specificities allied to the Ly-6 antigen. Secondly, we have investigated thymocyte heterogeneity using a combination of different physical separation techniques and cell surface marker analysis - in particular we have characterized various cell populations using Ly antisera and peanut agglutinin. These studies have led to the formulation of a theoretical model for the role of cell surface antigens in lymphocyte differentiation and migration.

117. SELECTIVE MANIPULATION OF T CELL SUBSETS BY ADJUVANTS

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Synthetic adjuvants will be utilized to selectively potentiate T cell subset functions directed against syngeneic tumor cells. These functions will be analyzed using distinct assays such as the generation of cytolytic T lymphocytes against syngeneic tumor cell surface antigens, amplification of the cytotoxic T cell (CTL) response by the helper T cell subset, and the generation of cytolytic antibody directed against syngeneic tumors. In addition, the potential of synthetic adjuvants to generate suppressor T cells which may regulate the activity of cytolytic T cells against tumors in vitro and in vivo will be assessed.

The two models chosen will focus on the period of tumor remission obtained between the time that the primary tumor is identified and the expected onset metastatic disease which occurs as the animals age. Immunotherapeutic protocols using highly activated subpopulations of T lymphocytes together with adjuvants will be tested. In addition, if adjuvants are found to induce suppressor cells in vivo, regimes will be undertaken to circumvent the induction of suppression in vivo. These protocols include thymectomizing the animal, pretreating the animal with cytoxan to reduce the possibility of the induction of suppression, and in vivo administration of anti Ig anti-serum.

These experiments should provide a rational basis for the utilization of adjuvants and specifically activated T cells in tumor-bearing mice.

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118. IMMUNOLOGY OF THY-1-BEARING LYMPHOCYTE SUBSETS

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Selective cytotoxicity of normal guinea pig serum affords a novel means for recognizing as well as functionally characterizing subsets of T lymphocytes. The phenomenon also lends itself to differential definition of cell membrane components of normal and tumoral lymphoid cells. Cytotoxicity in this system results from antibody-independent activation of the alternative complement pathway. This reaction will be exploited in this research to provide new insights into the ability of discrete lymphocyte subpopulations to undertake immunological functions in relation to expression (or lack of expression) of surface markers. The method is also suitable for studying membrane changes occurring during blastic transformation and proliferation of both normal and neoplastic lymphocytes.

Experimental approaches are designed to: a) Establish the capacity of discrete subsets of Thy-1-bearing rat lymphocytes to display typical T cell functions: b) define the ontogeny, distribution and kinetics of expression of GPS reactivity by normal lymphocytes; c) discriminate, on the basis of guinea pig complement reactivity, between cell surface characteristics (markers) of normal and malignant lymphoid cells; and d) establish traffic and homing patterns of lymphocytes seeking maturation through differentiation processes.

This investigation will contribute to a better understanding of the relative contributions of unique subsets of lymphocytes to the mounting of immune responses. It is also anticipated that differential properties of tumor cells will be more clearly defined by the results of these studies.

119. EXPRESSION OF IA ANTIGENS ON FUNCTIONAL CELL SUBPOPULATIONS

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It has been demonstrated that the T cell proliferative response to Con A and the T cell dependent IgM response to the soluble antigens TNP-KLH, TNP-T,G-(A-L), and TNP-nuclease require the participation of accessory cells. This subpopulation of spleen cells has been shown to be glass adherent, radioresistant, non-T, non-B and to express Ia (I region associated) determinants encoded in I-A and I-E/C. In addition, cells within the splenic adherent cell population are the predominant stimulators of the one-way murine mixed lymphocyte response when responder and stimulator cells differ either at H-2 or the Mls locus. These stimulator cells also bear I-A and I-E/C encoded determinants and their ability to stimulate allogeneic T cells could be inhibited with specific anti-Ia reagents. Similar results regarding Ia antigen expression and functional accessory cell competence were also obtained using a population of purified murine Kupffer cells, the tissue macrophage of the liver.

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120. PROTECTIVE CAPACITIES OF T-CELL SUBPOPULATIONS DIRECTED AGAINST LEUKEMIA

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Line Ib lymphocytic leukemia in C58 strain mice will be used as a model system in which to study the role of individual subpopulations of T-lymphocytes in the protective immune response to leukemia. Antisera to Lyt antigen and to Ia antigen markers will be used both as a primary means of separating populations and together with other methods as a probe to assess biophysical and pharmacological methods of cell separation. Using various effector cell-target cell combinations, the cytotoxic and cytostatic reactivity of purified lymphocytes on radioisotope labelled Ib leukemia cells will be determined. The influence of soluble antigens and of interaction between lymphocyte subpopulations will be assayed at the effector cell and target cell levels. In vitro reactions will be correlated with protection in vivo by quantitative adoptive transfer assays. Lyt markers will be monitored in vivo in an effort to show how changes in T-cell subpopulations are related to susceptibility and resistance to leukemia.

121. T CELL SUBSETS AND MAREK'S DISEASE VIRAL ONCOGENESIS

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Two highly inbred MHC-identical lines of chickens differ markedly in susceptibility to viral oncogenesis, and express contrasting T cell surface antigens determined by the independent Ly-4 and Th-1 loci. These antigens may be markers for partially overlapping subsets of T cells, and there is evidence that one of these loci, and perhaps both, are associated with resistance to Marek's disease oncogenesis. The extent to which these surface antigens are markers for distinct subsets of central and peripheral T lymphocytes will be analyzed during embryogenesis and in growing chickens, using immunofluorescent techniques and double labeling.

In vitro methods will be used to investigate whether the expression of suppressor and cytotoxic activity of immunocompetent cells is associated with the expression of inherited resistance to viral oncogenesis in Marek's disease, and attempts will be made to relate these functional properties to the T cell subsets.

122. SUBPOPULATIONS OF SENSITIZED T CELLS IN RATS

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No summary has been provided to the Smithsonian Science Information Exchange.

123. INTERACTION OF T CELLS IN IMMUNITY AND TOLERANCE

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This project will investigate the interactions of subpopulations of thymus-derived lymphocytes (T cells) in immunity and tolerance. Special emphasis will be given to a subset of naturally proliferating T cells in order to functionally characterize their role in various cell-mediated immune reactions, i.e., graft-versus-host reactions, mixed lymphocyte interactions, and responses to T cell mitogens, and to determine the relationship of these cells to classical long-lived, nonproliferating T cells. This project will also investigate the cellular mechanisms which control both the induction and expression of T cell tolerance in contact hypersensitivity to DNFB and TNCB in mice. These studies will emphasize (1) characterization of the cells involved, i.e., alloantigens such as Ia and Ly, (2) the genetic restrictions for their interaction, (3) identification and characterization of soluble factors, and (4) elucidation of the mechanism(s) of T cell-mediated immune regulation.

5. T CELL MARKERS, RECEPTORS, AND ANTIGENS

124. TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE (TDT) AS A DIFFERENTIATION MARKER IN NORMAL AND NEOPLASTIC HUMAN MONONUCLEAR CELLS

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Terminal deoxynucleotidyl transferase (TdT) is an enzyme which is a differentiation marker of a minor subpopulation of bone marrow cells and a major subpopulation of thymocytes. These bone marrow cells are thought to be, at least in part, prothymocytes. In hematopoietic neoplasms, TdT is found in the leukemic cells from a large majority of patients with acute lymphocytic leukemia and T cell lymphoblastic lymphoma. The enzyme is detectable in the blast cells of only a small minority of patients with acute granulocytic leukemia. The purpose of the proposed studies is the exploitation of this marker and the sensitive detection of minimal disease burdens in both non-T and T cell lymphocytic leukemia/lymphoma. A dual assay for T cell membrane markers and TdT has been developed which will be exploited in the detection of T cell lymphoblastic lymphoma cells in patients undergoing therapy for this disease. A dual assay for TdT and a membrane marker from the surface of non-T cell ALL cells is now being developed for application to the large sub-set of patients with ALL whose blast cells do not bear T cell markers. The goal of these studies is the early identification of recurrent disease after successful initial therapy. A final goal of these studies is the elucidation of the phenotype and developmental potential of the normal bone marrow cell that contains TdT. To achieve this goal, we would like to establish a correspondence between a cell membrane antigen and intracellular TdT in this minor subpopulation of normal bone marrow cells. The use of a fluorescence-activated cell sorter in the isolation of these marrow cells is planned.

125. ACUTE LYMPHOBLASTIC LEUKEMIA - AN IMMUNOLOGICAL STUDY OF A POSSIBLE T-CELL MALIGNANCY

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The application of T- and B-cell markers to the investigation of acute lymphoblastic leukemia (ALL) has resulted in an unmasking of the heterogeneity of the disease. One of these markers, human thymus/leukemia-associated antigen (HThy-L) has been studied, isolated, purified and characterized by the applicant in collaboration with Dr. E.W. Gelfand. Recently, we succeeded in developing a competitive radioimmunoassay for quantitation of HThy/L in cell extracts and plasma.

OBJECTIVE: This study was designed to define further the specificity of HThy-L and use this marker in investigations of pathways of T-cell differentiation in normal conditions and in leukemias.

APPROACH: Using a radioimmunoassay, we have demonstrated increased quantities of this antigen in leukemic cells and plasma from all patients with T-cell ALL and a considerable number of patients with other forms of acute leukemia. Neither healthy subjects nor patients with non-leukemic disease had increased quantities of HThy-L in peripheral blood mononuclear cells. The study of established hematopoietic cell lines has demonstrated that increased quantities of HThy-L are a more stable feature of leukemic T-cells than receptors for sheep erythrocytes (E). In this application, we propose to continue our studies on the clinical application of HThy-L, the relationship of the two forms of HThy-L with the pathway and stage of differentiation of normal and leukemic cells, and the biosynthesis and cellular localization of HThy-L and its structural characterization. The investigation of the acquisition of such cell markers at various stages of differentiation and of their role in differentiation may provide the means for diagnosing and monitoring ALL and for distinguishing or selecting more specific therapy for ALL of different origins.

126. LYMPHOCYTE MARKERS IN LYMPHATIC LEUKEMIA - POSSIBLE CLINICAL IMPLICATIONS AND STUDY ON SUBPOPULATION OF T LYMPHOCYTES

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The summary for this project is under entry no. 98.

127. TDT AS A MARKER OF UNDIFFERENTIATED T CELLS OF LEUKEMIA AND LYMPHOMAS

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This study aims at setting up immunological and biochemical methods in order to identify the presence of TdT in the cells of leukemic patients. According to the literature this marker seems to allow the identification of undifferentiated T lymphocytes and could be very important for the prognosis and therapy of hematological diseases.

TdT has already been extracted from 50 kg of calf thymus. At present 50 mg of purified enzyme is available. A new extraction is in progress in order to increase the amount of antigen. TdT purified after polymerization was emulsified in Freund adjuvant. Afterwards a group of rabbits was immunized by injections of antigen. The antibody was tested by immunodiffusion in agar phosphate buffer 7.3 pH against TdT. Antiserum produced a single sharp line of precipitation. We are now working at the purification of anti-TdT antibodies by passage on Sepharose-TdT column.

A method for biochemical enzymatic testing of TdT has been established. This kind of dosage must be tested in the cell extract of lymph nodes. TdT was tested measuring the incorporation of tritiated dGPT in insoluble acid matter. This method uses a poly(dA)55 which has been prepared in our laboratories. By means of this method 40 patients with lymphoblastic leukemia or lymphoma were examined. The enzyme has been revealed only in patients suffering from lymphoblastic leukemia or lymphoblastic lymphoma.

In the near future immunofluorescence and radioimmunological methods will be used in order to test leukemic or lymphomatous patients.

128. T-MICG AND N-MICG IN LYMPHOID MALIGNANCY

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The present studies aim 1) to examine the usefulness of T-cell derived and N-cell derived macromolecular insoluble cold globulin as markers for identification of cell types in human lymphoid malignancies; 2) to study the possibility that T-MICG and N-MICG may serve as prognostic indicators in acute leukemia; 3) to determine whether MICG is a differentiation marker. In vitro studies will make use of fluorescein conjugated antiserum to T-MICG and N-MICG and fluorescent microscopy to examine stained normal and malignant cells. Normal and neoplastic cells will also be radiolabelled in vitro to determine the distribution of MICG in normal and pathological lymphocytes.

129. T LYMPHOCYTES IN LYMPHOMA PATIENTS DETERMINED BY QUANTITATIVE AND SCORING EVALUATION OF ROSETTE FORMATION

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OBJECTIVES: Study of receptors for sheep red blood cells (SRBC) present on T lymphocytes in lymphomas, principally in Hodgkin's disease (H) patients, who are known to have impaired lymphocyte function. Studies made by Khan et al. (Cancer Res. Proc. 16:65,75) show a modification of T lymphocytes after in vitro treatment with transfer factor. In our study, we adopted the scoring technique for quantitative evaluation of T lymphocyte receptors in untreated H and non-Hodgkin's (n-H) lymphomas, during and after radio- and/or chemotherapy.

APPROACH: Blood was collected from normal donors and lymphoma patients and lymphocytes separated on ficoll-hypaque gradient. Lymphocytes washed with isotonic saline solution were resuspended in Hank's solution to obtain a 4 times

10 to the 6th power/ml concentration. SRBC washed twice with isotonic saline solution were resuspended in Hank's solution at 8 times 10 to the 7th power/ml concentration. Transfer factor (TF), prepared from peripheral lymphocytes, one unit (1 ml) corresponding to 10 to the 8th power lymphocytes, was diluted 1:50 before use. Lymphocyte and SRBC suspensions, 0.25 ml of each, were mixed and incubated for 15 min. at 37 degrees C, centrifuged and left overnight at 4 degrees C. Effect of TF was evaluated by adding 0.025 ml of 1:50 diluted TF to the lymphocyte suspension just before addition of SRBC and incubation. All samples were evaluated by counting percentages and by scoring. For scoring, the SRBC attached to each lymphocyte were counted, using the following criteria: SRBC 0-2, 3-5, 6-8, 9-11, 12 or more corresponding to 0, 1 plus, 2 plus, 3 plus and 4 plus, respectively.

RESULTS: Ten normal donors show no statistically significant differences in percentages and scores either before (59.5% plus or minus 4.8/127.9 plus or minus 8.87) or after addition of TF (64.0% plus or minus 5.5/142.9 plus or minus 13.0).H patients' (10 cases) lymphocytes resulted in 44.1% plus or minus 9.5/79.8 plus or minus 18.56 before and 59.6% plus or minus 7.77/112.6 plus or minus 18.65 after addition of TF, the differences being statistically highly significant (p less than 0.01). (Text Abridged.)

130. STUDY OF T-LYMPHOCYTE FUNCTION IN MALIGNANT LYMPHOMAS

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OBJECTIVE: To isolate T-lymphocytes from malignant lymphomas and to study their functional characteristics.

APPROACH: Hodgkin's and non-Hodgkin's malignant lymphomas are obtained fresh as a result of biopsy procedures or staging laparotomies. Lymphocyte populations are isolated and separated by rosetting with sheep erythrocytes and differential centrifugation. T-lymphocytes may be further divided on the basis of Fc gamma and Fc mu surface receptors. The functional characteristics of these cells will be assessed as follows: a) Helper or suppressor activity when added to pokeweed mitogen stimulated B-lymphocytes. Assessed by measuring plasma cell differentiation. b) Cytotoxicity against autochthonous lymphoma cells and various target cell lines. Measured by chromium release.

131. CHARACTERIZATION OF ANTIGEN-BINDING T-CELL RECEPTORS

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OBJECTIVE: T-cell tumors are a model for normal T-cells in the sense that they express similar surface markers. As the molecular structure of the T-cell receptor for antigen is still unknown, we would like to find out whether antigen-specific receptors can be found on murine T-cell tumors. This would yield enough receptor to make chemical analysis possible.

APPROACH: As normal T cells activated by a mixed lymphocyte reaction bind alloantigens from stimulator cells and expose receptors which specifically react with anti-receptor-antibodies, we would like to apply similar tests to the screening of T-cell tumors for antigen-specific receptor expression.

132. COMPARISON OF HELPER AND SUPPRESSOR T CELL RECEPTORS

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The summary for this project is under entry no. 54.

133. THE ARRANGEMENT OF GENES ENCODING ANTIGEN-BINDING RECEPTORS IN T CELLS

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The structural characteristics and genetic mechanism which generates diversity of Ag-binding receptors on T cells is the proposed area of investigation in this fellowship application. Various lines of evidence suggest that Ag-binding receptors on T cells have properties similar to those of serum immunoglobulin (Ig). Unlike Ig, however, Ag binding receptors on T cells have been difficult to purify and biochemically characterize. We propose to investigate DNA sequences which are complementary to mRNA encoding Ig-like molecules isolated from T cells. Using a continuous T cell line bearing a known idiotype specificity should enable us to purify mRNA encoding Ig sequences and to correlate the translation product of that mRNA to the Ag-binding receptor of the T cell line. In myeloma cells, variable (V) and constant (C) region genes are recombined to generate an actively transcribed DNA segment. We propose to determine whether similar events have occurred in the putative V and C region genes of a T cell line.

134. SPECIFICITY OF ANTIGEN-BINDING RECEPTORS ON T-CELLS

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OBJECTIVE: A body of evidence suggests that humoral antibodies of a certain specificity, e.g., antistreptococcal A polysaccharide, share V domain antigens with T cell antibodies of the same animal. We want to study whether T and B cell antibodies also share fine-specificity characteristics in antihapten; the next step is to determine whether or not the same fine-specificity 'idiotype' is present in T cell antibodies.

APPROACH: Inherited fine-specificity idiotypes were found in several antibodies and all were controlled by an allotype-linked gene (VH). Two look promising for the proposed study. One is anti-NP (3-nitro-4-hydroxyphenyl acetic acid) and the other is anti-ABA-HOP (para-azobenzenearsonate coupled to carbon atom 5 of parahydroxyphenyl acetic acid). The promising fact is that the anti-NP idiotype of C57BL/6 and LP strains is strongly heteroclitic (affinity for NNP is ten times higher than affinity for the immunizing hapten NP), and the anti-ABA-HOP of RIII and C57BL/6 strains is similarly heteroclitic (affinity for ABA-MIP is twenty times higher than affinity for ABA-HOP).

135. ISOLATION AND CHARACTERIZATION OF T CELLS AND NORMAL CELL RECEPTORS

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OBJECTIVES: Isolate and characterize T lymphoma cell and normal T cell receptors for T-MuLV's, and define their specificity in terms of the molecules and determinants that they recognize, as well as determine whether they belong to a single class of cell surface receptors or several different kinds of receptors. We shall use techniques used for the isolation of cell surface receptors for defined ligands such as antigens (the receptors are immunoglobulins), hormones, neurotransmitters, and specific cell-surface antigens. Since the fibrotropic retroviruses in chickens and mice bind to fibroblasts by a viral envelop protein-cell surface receptor interaction, both lymphoid and fibroblast receptors will be isolated. We propose that the cell-surface receptors in fibroblasts are absent from most, if not all, thymocytes and thymic lymphoma cells; a test of this hypothesis will require an extensive comparison of type-specific and general receptors for the same ligands - the T-MuLV envelop glycoproteins (18-21).

136. BIOCHEMICAL CHARACTERIZATION OF T-CELL RECEPTOR

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The objective of this research is to isolate and characterize the T-cell receptor for antigen. Antigen-binding T cells from the spleens of bursectomized, agammaglobulinemic chickens can be enriched by adherence to plates of antigen-derivatized gelatin. T lymphocytes enriched in cells capable of binding to the antigen human gamma globulin (HGG) will be labeled biosynthetically with 35S-methionine, incubated with HGG to allow for the formation of antigen-receptor complex, and lysed. The HGG-binding material will be isolated by affinity chromatography on Protein A-Sepharose and analyzed by SDS-polyacrylamide gel electrophoresis and autofluorography. There is preliminary evidence that one of the proteins detected in this manner may be T cell receptor. Experiments to test for the specificity of this putative antigen receptor will be performed. If the results confirm its identity, the protein will be analyzed as follows: (1) Serological analysis will be performed to test for the presence of immunoglobulin constant region or major histocompatibility determinants on the molecule. (2) Incorporation of 14C-fucose will reveal whether the receptor is a glycoprotein. (3) Partial proteolysis and peptide mapping will reveal any amino acid sequences held in common with immunoglobulin heavy chains.

137. STUDIES ON THE POLYMORPHISM OF ANTI-MHC T CELL RECEPTORS

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One finding of interest that emerges from the studies of anti-MHC T cell receptors using anti-idiotype antibodies is that these receptors appear to be polymorphic. Using a different assay, namely the specific suppression of lethal graft versus host reactions (GVH) by T cell populations expressing anti-anti-MHC properties I have accumulated data which suggest that anti-MHC receptors on T cells display only a very limited degree of polymorphism. These results raise many interesting questions. Is it that allo-anti-idiotypic antisera detect some polymorphism and are these determinants on anti-MHC receptors different from those which trigger T cell responses? The approach to be used to answer these and related questions will be to obtain T cell populations from a variety of different rat strains reactive to a particular MHC haplotype and examine them for their idiotypic expression defined by hetero- and allo-anti-idiotypic antisera. Purified T cell receptor material shed from cloned alloreactive T cells will constitute the primary source of immunogen. The availability of anti-idiotypic reagents and the technology to exploit them in Uppsala will permit a definitive serological characterization of the extent of polymorphic diversity of anti-MHC receptors in rats. If these studies detect non-polymorphism it may have some significance for MHC restricted T cell specificity in the anti-self repertoire.

138. ANALYSIS OF T CELL ALLOANTIGEN RECEPTORS USING MONOCLONAL ANTIBODIES

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OBJECTIVE: To investigate the nature and specificity of T lymphocyte antigen receptors. Two main problems will be focused upon: first, the role of mouse H-2-linked immune response (Ir) genes in T cell mediated antigen recognition and second, the role of idiotypic determinants in T cell recognition of H-2-linked immune response (I) region associated (Ia) histocompatibility alloantigens.

APPROACH: These two problems will be addressed using hybridoma anti-Ia and anti-idiotype antibodies. A monoclonal anti-Ia antibody, which was recently derived in my laboratory, detects an Ia molecule encoded by two distinct complementing Ia loci and displays a strain distribution analysis very similar to that of the two H-2-linked complementing Ir genes that regulate an immune response to pigeon cytochrome C. This antibody will be tested for its capacity to inhibit an in vitro T cell proliferative response to this antigen. Such an inhibitory capacity might establish a definite molecular interrelationship between Ir gene products and Ia antigens.

Monoclonal anti-idiotypic antibodies that are reactive with T cell alloantigen receptors will be generated by immunization of mice with either monoclonal anti-Ia antibodies or alloactivated T cell blasts. Monoclonal anti-idiotypic reagents will be used in combination with monoclonal anti-Ia antibodies to select for and analyze functional subpopulations of alloactivated T cells which express either particular surface idiotypic determinants or Ia alloantigen receptors, or both of these structures. These studies should provide a better understanding of the idiotypic diversity of T cell receptors for alloantigens.

139. MEMBRANE BINDING TO T LYMPHOCYTE RECEPTORS FOR ALLOANTIGENS

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We are attempting to analyze the cell-cell interaction between killer T-lymphocytes and allogeneic target cells. Since it is clear that this occurs via receptors on the T lymphocyte for H-2 antigen, we have attempted to study the binding of plasma membrane vesicles bearing these antigens to the killer lymphocytes. Although specific binding of the vesicles can be detected by indirect immunofluorescence, quantitative measurements using radioactive vesicles fail to give evidence for specific binding. Furthermore, no specific inhibition of cytotoxicity was achieved by the vesicles even when aggregated into a multivalent assay. However the same amount of antigen on live 'cold' target cells very efficiently blocks cytotoxicity in a specific fashion. At this stage we can only conclude that H-2 antigen alone is not adequate to establish a strong binding to the killer T cell receptor for alloantigen.

140. MURINE T LYMPHOCYTE RECEPTORS FOR ALLOANTIGENS

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The objective of this project is to raise and characterize an antibody directed against the combining site of a T lymphocyte receptor for a cell surface antigen. Such an antireceptor antibody will be used to establish a murine model for studying the genetics and cellular interactions involved in the cellular immune response to tumor antigens and transplantation antigens. Cloned cytotoxic T lymphocytes will be used for immunization and as a target for assaying the antibody. The cytotoxic cells will be generated to recognize the difference between a mouse strain and a histoincompatible strain differing from the first only in a point mutation. The principal criterion of receptor specificity of the antibody produced will be preferential activity after appropriate absorptions for cytotoxic cells of the original specificity. The antibody will be used to test the following hypotheses: (1) the combining site is genetically determined and linked to the major histocompatibility complex or to IgG heavy chain allotype, (2) the same combining site is found on both B and T cells, (3) the combining site can be detected in animals expressing the antigen with which the combining site interacts, (4) the antibody can induce an antigen specific immune response in the absence of antigen or suppress the immunoresponse to antigen. These hypotheses bear in fundamentally important ways on the specific interactions of the immune response with tumor antigens and transplantation antigens and would lead to predictions concerning clinically useful manipulations of the immune response to these antigens.

141. INDUCTION AND REGULATION OF IMMUNITY

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The role of mouse T lymphocytes bearing a receptor for the Fc portion of IgM is investigated. So far cells with helper function for antibody producing B cells have been found positive for this marker. The receptor is also involved in antibody dependent, cell mediated cytotoxicity with IgM antibody. Further functional studies including reactivity to mitogens and allograft reactivity are in progress.

The target structure for natural killer cells in the mouse is investigated using 1) IgG from normal rabbit serum, which have antibody with the same specificity pattern as natural killer cells, and 2) biochemical analysis of the oligosaccharides extracted from the membranes of tumor target cells, since those oligosaccharides strongly inhibit the natural killer system.

Using a combination of specific antisera and fractionation of tumor cells in anti-Ig columns we select antigenic loss variants. Studies on the transplantability of those variants in allogeneic mice or in mice specifically immune to tumors are performed in order to clarify the relative roles played by serologically defined tumor antigens and antigens responsible for cell mediated tumor rejection.

The immunogenetics of the immune response of mice to a synthetic antigen (PVP) is investigated using isoelectric focusing of specific antibodies and antiidiotypic antisera. Some mouse strains classified as high responders have Ig-1 allotype linked gene(s) determining high response.

142. T-CELL RECEPTOR AND EFFECTOR MOLECULES

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The mechanism of cell lysis by cytotoxic T lymphocytes (CTL) has been examined using both rabbit and mouse CTL. A standard murine (CBA or BALB/c anti-EL4) CTL system was used to demonstrate that cell surface thiols and proteases were necessary for CTL function (Redelman and Hudig, J. Immunol., 124, in press, 1980). Concurrently, a rabbit anti-mouse CTL system was developed to take advantage of the unique Ig VH allotype system of the rabbit that would allow one to identify receptor framework determinants. Rabbit cytotoxic effector cells were generated by culturing rabbit lymphoid cells from the spleen, mesenteric lymph nodes (MLN) or peripheral blood (PBL) with mitomycin-treated mouse stimulators for 4-7 days. These cytotoxic cells had the following properties of CTL: Rabbit T cells were necessary and sufficient for their generation, the effector cells could distinguish between targets of different inbred mouse strains including congenics differing only at H-2, both tumor and normal mitogen-stimulated blasts could be lysed and lysis was not inhibited by aggregated Ig. The generation of these CTL could be modulated by cells with properties resembling B and/or plasma cells (Russell and Redelman, Fed. Proc., 38, 1016, 1979). Lysis by these CTL could also be inhibited by specific anti-T cell serum (ATS) in the absence of exogenous complement (Redelman and Trefts, J. Immunol., 121, 1532, 1978). Future experiments will seek to determine whether rabbit CTL express Ig VH determinants and the identity of the functional molecule(s) recognized by ATS. It is possible that the ATS may inhibit by interfering with molecules analagous to those with thiols and/or protease activity that were demonstrated in the mouse CTL system.

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143. IDENTIFICATION OF DIFFERENTIATION ANTIGENS ON HUMAN LYMPHOCYTES

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We propose to prepare a series of hybrid cell lines that produce monoclonal antibodies to human lymphocytes. Our primary goal is to obtain monoclonal antibodies specific for T cells. Fusion experiments will be performed with mouse spleen cells immune to normal T cells, activated T cells, thymocytes, and cultured leukemic cell lines. Our strategy for selecting specific hybrid clones will be to utilize pairs of matched or autologus normal or activated lymphoid cells, or leukemic lines. These reagents will be used to identify functionally distinct subpopulations of T cells, especially cytotoxic T lymphocytes and their precursors, helper cells and suppressor cells. Monoclonal antibodies obtained will be used to study differentiation antigens as they are expressed by cultured hematopoietic cell lines, cultured leukemic lines and leukemic blasts. Clinically, we will use these reagents to characterize the alterations occurring in the lymphocyte subpopulations of patients with acute and chronic GVH disease. One of the central objectives will be to explore the feasibility of using monoclonal T cell antibodies for the prophylaxis or treatment of GVH disease.

144. AN INVESTIGATION OF THE LYT SURFACE COMPONENTS OF T LYMPHOCYTES

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The Lyt system of T-lymphocyte antigens is unique among the known antigenic markers for T-cells in being expressed differently on different functional subclasses of T-cells. 'Helper' cells express the Ly-1 specificity whereas 'killer' and 'suppressor' cells express the Ly-2, 3 specificities. It is not yet clear whether the Lyt components function only as differentiation markers for these populations or whether they are actually involved in T-cell function.

This project will direct itself to that question, on the assumption that a biochemical characterization of T-cell membranes is an essential part of the solution. Purified pools of helper and killer/suppressor cells will be obtained using cytotoxic Ly antisera and their surface membranes will be characterized. The associations of Ly components with other defined components in the T-cell membrane will be explored, with particular reference to the receptors for alloantigen that are expressed by both helper (Ly-1) and killer (Ly-2,3) cells after stimulation in mixed lymphocyte culture. A biochemical analysis of the Lyt components after isolation from the membrane will also be attempted.

The results obtained should elucidate some of the modes of interaction of T-cells with one another, with B-cells, and with antigen.

145. MEMBRANE SPECIFICITIES RELATED TO NEOPLASIA

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The purpose of this investigation is to define the molecular nature of the murine cell surface components Thy-1, H-2 and GIX (gp70). Thy-1 and GIX are expressed on T-lymphocytes and are indicative of their state of differentiation. GIX is also related to MuLV glycoproteins and expressed on thymus derived leukemias. These components will be obtained from murine lymphoblastoid cell lines grown in 70 and 200 liter culture vessels. Plasma membranes from the cell lines will be purified and the components solubilized with detergents. The solubilized Thy-1, H-2 and GIX will then be purified by a combination of techniques including lectin affinity chromatography, gel filtration, ion exchange chromatography and isoelectric focusing. These procedures have been utilized to great advantage in the isolation of murine Thy-1.1 and Thy-1.2. The purified components will be physicochemically characterized by determining their molecular weight, amino acid and carbohydrate compositions and the existence of subunits. Thy-1 has been isolated in quantities sufficient to allow limited structural analysis of the sequence of amino acids. Providing H-2 and GIX can be isolated in sufficient quantities, these molecules will also be analyzed in a limited degree with regard to their primary structure. On the basis of these studies one should be able to make deductions regarding the differences in specificities expressed on leukemic cells as opposed to normal ones. It is anticipated that knowledge concerning the molecular nature of cell surface components will be of significance in clarifying the normal differentiation process as well as the nature of cancer. Moreover, structural data on these molecules should aid in a better understanding of the molecular nature of lymphocyte membrane components.

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6. T CELL SPECIFICITY, RECOGNITION, AND ANTIGEN SENSITIZATION

146. SPECIFIC HOST CELL RECOGNITION IN TUMOR IMMUNITY - MODEL EXPERIMENTS WITH ALLOREACTIVE T CELLS

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OBJECTIVE: Characterization of the specificity of alloantigen recognition by T cells activated in the mixed lympholyte reaction (MLR). Relation of alloantigen-binding T cells to different functional T cell subsets. The ontogeny of alloantigen-recognizing T cells.

APPROACH: Alloantigen binding to MLR-activated cells by using different sources of subcellular H-2 antigen material (membrane vesicles, MLR supernatants, soluble H-2 antigens). Analysis of binding specificity by antibody inhibition using antisera with restricted specificity to H-2 determinants. Alloantigen binding to isolated cells bearing Ly and Qa markers. Detection of specific alloantigen binding by immature thymocytes.

PROGRESS REPORT: We have quantitated alloantigen binding to MLR activated T blasts by using radiolabelled allogeneic membrane vesicles as a source of antigen. Furthermore, recent experiments have indicated that prolonged survival of stimulation cells (using mitomycin treated stimulations) provides MLR supernatants with a good source of antigens, which bind specifically up to 95 percent of T blasts. Preliminary experiments suggest that the binding can be inhibited by anti-H-2 sera directed against stimulation determinants. These findings make it possible to analyze specificity and cross-reactivity of alloantigen receptors at the single cell level.

Recent experiments in this laboratory have indicated that cells incubated with a variety of antibodies to surface determinants form rosettes with protein-A coated sheep erythrocytes, and the rosettes can be isolated by Ficoll gradient centrifugation. This isolation procedure will be applied in the study of antigen binding to different T cell subsets.

We have shown that radiolabelled membrane vesicles bind to a detectably large proportion of thymocytes. This finding will be exploited in studying alloantigen binding in relation to T cell ontogeny.

147. T-CELL RECOGNITION AND RESPONSIVENESS IN CLONED CONTINUOUS CELL LINES

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The objective of this research proposal is to determine the nature of the recognition processes in thymus dependent lymphocytes. To study antigen recognition of a single specificity from the heterogeneous milieu of a normal population of T-cells, cloned T-cell lines will be derived and propagated in culture. The immunologic function and antigen specificity of T-cell clones will be studied by measuring the ability of these clones to function as T-helper-cells in the T-cell dependent induction of antibody synthesis. In order to be able to study specific determinant recognition, the species variants of cytochrome c molecules will be used as antigen. Preliminary results indicate that after multiple stimulations with erythrocyte antigens, T-cell lines become highly enriched for antigen specific T-helper-cells. The use of well-characterized antigens and cloning techniques should provide a defined system for studying T-cell recognition.

148. NATURE OF T CELL AND MACROPHAGE FACTORS (MEDIATORS OF CELL INTERACTION)

Feldmann, M., Cecka, M., Winger, L., Kontiainen, S., Simpson, E., Todd, I., Erb, P., Cosenza, H., Imperial Cancer Research Fund, Tumor Immunology Unit, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

Biochemical analysis of T cell factors has yielded encouraging results: biosynthetically labelled T cell hybridomas release labelled antigen-binding material which has been analyzed on gels following elution from antigen columns. Several sets of peaks were detected, ranging in molecular weight from approximately 70,000 to 25-30,000 daltons. The detailed patterns varied from run to run, and with various hybridomas, but the data are most compatible with a two-chain model of factor structure, the patterns being complicated by proteolytic breakdown. Attempts are in progress to reduce proteolytic breakdown and to resolve the number of chains involved and the location of the serologically detected determinants on these chains.

As there are several reports of T cell receptors sharing idiotypic determinants with antibody molecules, it is likely that the T cell receptor uses immunoglobulin variable region products for antigen recognition. It was thus of interest to examine the antigen-combining site of T cell factors for the presence of immunoglobulin idiotypes. We have used several antigenic systems: phosphorylcholine (Pc) in BALB/c mice, nitrophenyl in C57BL/6 mice, systems in which the antibody response has major idiotypes. Helper factor to Pc in BALB/c mice reacted with anti-idiotype antisera, indicating that T cell factors have the same or similar combining site as T cell receptors and B cells.

Antisera have been prepared by Dr. D. Givol (Weizmann Institute, Rehovot, Israel) against the antigen-combining fragment of a myeloma protein, MOPC 315, which react with framework determinants of the combining site of the majority of heavy chains. Such antisera (anti-VH) react with helper T cell factors, but not anti-VL antisera prepared from the light chain variable region of the same myeloma. This reinforces the concept that the combining site is an Ig variable region. The rest of the factor molecule contains I region determinants and 'constant' region determinants, as defined by rabbit antisera. These rabbit antisera to mouse factors also recognize monkey and human factors of the same type, indicating that portions of these molecules are highly conserved. The genetic origin of the C region is unclear, but in view of the fact that there is a variable region which is Ig in nature, we are testing the hypothesis that the C region may also be Ig-linked.

149. SPECIFIC HOST CELL RECOGNITION IN TUMOR IMMUNITY

Elliott, B. E., Nagy, Z., Takacs, B., Queen's University, Dept. of Pathology, *King St. W., Kingston, Ontario,* K7L 3N6, Canada

OBJECTIVES: 1) To characterize the recognition structures involved in specific host cell recognition. 2) To determine the nature of antigenic determinants recognized by host cells and the association of such antigenic determinants with MHC gene products.

APPROACH: 1) To obtain populations of T cells enriched for their ability to react specifically with stimulator determinants. 2) To detect specific binding of radiolabelled stimulator membrane vesicles by MLR-activated T cells. 3) To characterize serologically and biochemically the T cell recognition structure.

PROGRESS: An assay was developed to quantitate binding of rediolabelled membrane vesicles by T cells activated in the primary mixed lymphocyte reaction. T blasts generated in unidirectional reciprocal MLR combinations were found to bind much more effectively membrane vesicles prepared by nitrogen cavitation from allogeneic stimulator than from syngeneic spleen and lymph node cells which have been previously labelled biosynthetically with 3H-leucine. Specific inhibition of binding with cold vesicles prepared from stimulator cells but not from responder cells was observed. The number of specific antigen binding cells is proportional to the concentration of membrane vesicles used. The proportion of labelled cells increases between 15 and 60 minutes of incubation with membrane vesicles, but thereafter remains constant. The binding of stimulator material is H-2 specific: cells bind membrane vesicles from congenic mouse strains only if they share the H-2 antigen of the original stimulator strain. Responder blasts bind labelled stimulator membrane fragments after as early as 2 days of primary MLR culture; no binding of membrane vesicle by small lymphocytes was detected between 2 and 5 days of culture. However, between 8 and 12 days of culture a significant proportion of small lymphocytes bind specifically stimulator membrane vesicles. The recognition function is sensitive to trypsin treatment but is regenerated within 5 to 6 hours. By this approach, binding of radiolabelled vesicles is quantitated independently of alloantibodies, and binding of syngeneic material can be assessed.

150. RECOGNITION AND KILLING BY CYTOTOXIC T LYMPHOCYTES

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The summary for this project is under entry no. 80.

151. MOLECULAR BASIS OF CYTOTOXIC T CELL RECOGNITION

Stallcup, K. C., Harvard University, Boston Campus, School of Medicine, Dept. of Pathology, *25 Shattuck St., Boston, Massachusetts,* 02115, United States of America

Murine cytotoxic T lymphocytes (CTL) specifically recognize and kill cells infected with virus. Killing of the infected cell by the CTL involves recognition of both viral protein(s) and the H-2 antigen products of the host major histocompatibility complex. Liposomes containing partially purified H-2 antigens and Sendai virus membrane proteins are known to stimulate specific

CTL. The liposome system will be utilized to biochemically define those viral proteins which are recognized in the immune response. Deglycosylated viral and host glycoproteins will be assayed for biological activity, to determine the contribution of sugars to antigenicity. Experiments will be undertaken to attempt to isolate a biologically active complex consisting of both viral and H-2 antigens, and any complex identified will be biochemically characterized.

152. T-CELL RECOGNITION OF THE INFLUENZA VIRUS INFECTED CELL

Freeman, G. J., Sidney Farber Cancer Center Inc., Division of Tumor Immunology, *44 Binney St., Boston, Massachusetts,* 02115, United States of America

I propose to study T-cell recognition of the influenza virus infected cell. The cytotoxic T-lymphocyte (CTL) response to the influenza virus infected cell is primarily directed against the viral M protein in association with H-2. The binding specificity of the immune CTL will be directly examined using a quantitative binding assay of radiolabelled membrane vesicles bound by CTL cells. Vesicles containing combinations of H-2 and viral antigens will be employed. Viral immune CTL will be cloned and propagated using T-cell growth factor. Cloned CTL cells will be used in the analysis and have the advantage of permitting a direct assay of the binding specificity of a uniform population. This approach should test whether the T-cell receptor recognizes the virally infected cell with a single or dual receptor.

Antibody to T-cell receptor for antigen will be prepared by using viral immune CTL clones as immunogen. Hybridomas will be prepared from the immunized mice and appropriate screenings for anti-T-cell receptor antibody will be used in the purification and characterization of the receptor.

I will examine whether the T-cell receptor for virally infected cells shares part of the recognition structure used by viral antibody. Using antisera against the idiotypic determinants of anti-M antibody, I will examine whether CTL cells share idiotypic determinants with the B cell. This will be done by analyzing the specific binding of radiolabelled anti-idiotype by various viral immune T cells.

153. T-LYMPHOCYTE RECOGNITION OF VIRUS-INFECTED CELLS

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This research will analyze mechanisms of immune surveillance of virus-infected cells in mice. Specifically, the action of thymus-derived lymphocytes (T-cells) in the immune killing of histocompatible, virus-infected target cells will be studied. Such studies of T-cell killing of infected histocompatible cells in mice are of significance in understanding immune surveillance of malignant cells in man.

Cytotoxic T-cells and virus-infected target cells will be generated using defined strains of Type A and B influenza virus. Cell-cell interactions will be assayed in vitro. Biochemical analyses and radioimmunoassays will be performed to characterize the nature of the cell surface receptors involved in the immune surveillance phenomenon.

This proposed project has four immediate aims: (1) to elucidate the mechanisms of induction of cytotoxic T-cells, (2) to study their role in defense against virus infection and altered self, (3) to learn the relationship between circulating antibodies and the induction of the cytotoxic T-cells, and (4) to characterize their cell recognition receptor molecules.

154. T CELL RECOGNITION IN THE MIXED LYMPHOCYTE RESPONSE

Hodes, R. J., Nadler, P. I., Gress, R., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Immunotherapy Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

The ability of subpopulations of murine spleen cells to stimulate a mixed lymphocyte response (MLR) was studied. It was found that T cells and B cells were poor stimulators of an MLR across H-2 or Mls differences. However, a non-T, radiation resistant splenic adherent cell (SAC) population was up to 20-50 times more efficient as a stimulator of these MLR's on a per cell basis than an unseparated spleen population. These SAC were shown to express Ia (I-region associated) determinants encoded by genes in I-A and I-E/C. These results suggest that Ia-positive SAC may be the predominant stimulating cells in spleen cell populations, and the preferential target for T cell recognition in cell interaction events. It has further been investigated whether T cells responding to Mls determinants recognize such determinants in the context of 'self' H-2 determinants. Studies employing limiting dilution techniques suggest that T cell responses to Mls involve the recognition of Mls in association with H-2.

BIBLIOGRAPHIC REFERENCES: Ahmann, G.B., Nadler, P.I., Birnkrant, A., and Hodes, R.J.: T cell recognition in the mixed lymphocyte response. I. Non-T, radiation resistant splenic adherent cells are the predominant stimulators in the murine mixed lymphocyte reaction. J. Immunol. 123: 903-909, 1979.

155. LYMPHOCYTE RESPONSES TO SYNGENEIC ANTIGENS

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One of the most compelling problems in immunology is an understanding of the pathogenesis resulting from lymphocyte recognition of altered-self determinants. Immunologic mechanisms underlie many autoimmune diseases and have also been implicated as promoters of lymphoid cell neoplasia. SJL/J mice offer a unique opportunity to dissect the cellular requirements and sequelae associated with T cells recognition of syngeneic B cell surface antigens. SJL T cells proliferate in vitro when they are co-cultured with autologous B cells. In addition, the B lymphocyte-derived reticulum cell sarcomas (RCS), which occur in high incidence in this strain, express Ia antigens that appear to elicit a vigorous proliferative response in syngeneic thymus, lymph node or spleen cells. T cell proliferation can also be observed in vivo following injection of irradiated RCS cells into syngeneic recipients. Experiments will be undertaken to determine whether the T cell response to syngeneic B cells or to B cell derived RCS tumors initiates cellular interactions that promote autoimmune disease and trigger the appearance of neoplastic lesions in syngeneic hosts. This hypothesis will depend on demonstrating: (1) that responding T cells produce soluble mediators (lymphokines) that affect host bystander cells, (2) that under the influence of responding T cells, host B lymphocytes are recruited for the production of autoreactive antibodies, and (3) that the T cell responses to syngeneic determinants precipitate the appearance of B cell lymphomas of host origin. Detailed analysis of T cell reactivity to syngeneic antigens will provide an appropriate foundation for future investigations to elucidate agents that may cause subtle self-alterations which induce autoresponsiveness and to determine how the recruitment of bystander cells promotes oncogenesis.

BIBLIOGRAPHIC REFERENCES: Ponzio, N.M., Chapman-Alexander, J., and Thorbecke, G.J. Properties of reticulum cell sarcomas in SJL/J mice. VI. Characterization of lymphoid cells that proliferate in response to RCS cells. Cell. Immunol. 41:, 1978, in press.

156. CHARACTERIZATION AND IN VITRO GROWTH OF ANTIGEN SPECIFIC HUMAN T LYMPHOCYTES

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The objectives of this project are to grow antigen specific human T lymphocytes in long-term tissue culture and to characterize both the specific effector cell which is grown and the feeder cell which is required to maintain growth. Ultimately it may be possible to use continuously cultured cells to raise and characterize antisera which define functionally distinct subclasses of human lymphocytes.

At least two T cell activators can be used to induce growth in human T lymphocyte populations. Lymphoblastoid cells and phytohemagglutinin activate one population of cells to produce a factor which then stimulates growth in the cytotoxic subpopulation. Mixtures of these two mitogenic stimuli are more effective growth inducers than either alone. Using a xenogeneic (human anti-mouse) model as the source of antigen specific T lymphocytes we have been able to grow specific cells for at least six months. We intend to apply this technique to growing clones of specific cells selected either by gravity sedimentation or by soft agar cloning. Ultimately we will attempt to grow selected tumor specific cells from patients with malignant disease.

157. PROPAGATION OF THYMUS-DERIVED LYMPHOCYTE LINES

Ruddle, N. H., Zuniga, M. C., Yale University, School of Medicine, Dept. of Epidemiology & Public Health, *333 Cedar St., New Haven, Connecticut,* 06510, United States of America

The overall goal of this project is an in-depth analysis of the thymus derived lymphocyte and its products. The basic approach is to use continuously propagable T cell lines which express specificity for individual antigens. Highly purified, functionally characterized, antigen sensitized T cells will be fused with extensively analyzed, cloned T cell lymphomas to make T cell hybrids. Lymphoma parents will be chosen which most nearly approximate the differentiated state of the primary T cell. In other experiments purified T cells will be treated with antigen and T cell growth factor. Antigen-specific continuous T cell lines will be used as source material to analyze the parameters which regulate T cell recognition of antigen. T cell lines will be used as source material for molecular analysis of lymphokines and T cell receptors by SDS-polyacrylamide gel electrophoresis, column chromatography, and two-dimensional gel electrophoresis.

BIBLIOGRAPHIC REFERENCES: Ruddle, N.H. Delayed hypersensitivity to soluble antigens in mice II. Analysis in vivo. Int. Arch. Allergy Appl. Immunol. 58: 44-52, 1979. Ruddle, N.H., Eardley, D., O'Connor, S., and R.E. Cone. Expression of antigen binding activity in T lymphocyte hybrids. J. Supramolecular Structure Suppl. 3: 303, 1979.

158. LYMPHOCYTES AND HISTOCOMPATIBILITY ANTIGENS

Wilson, D. B., Sprent, J., University of Pennsylvania, School of Medicine, Dept. of Pathology, *36th & Hamilton Walk, Philadelphia, Pennsylvania,* 19174, United States of America

Recently, procedures have been developed for the selective enrichment (positive selection) and depletion (negative selection) of alloantigen-reactive lymphocytes. These procedures will be applied to two questions concerning the biological function of gene products of the major histocompatibility complex (MHC) and the high frequency of MHC alloantigen reactive T lymphocytes: (1) The extent of involvement of MHC gene products in the induction and expression of certain T cell mediated immune functions, namely cytotoxicity, delayed-tvpe hypersensitivity and helper responses for B cells; and (2) the possible involvement of anti-MHC receptors present on the majority of T cells in the specificity of triggering with conventional antigens.

These questions have a direct bearing on the mechanism of T-cell stimulation by antigen and on whether T cells possess multiple specificities for antigen.

BIBLIOGRAPHIC REFERENCES: R. Korngold and J. Sprent. Selection of cytotoxic T-cell precursors specific for minor histocompatibility determinants. I. Negative selection across H-2 barriers induced with disrupted cells but not with glutaraldehyde-treated cells: Evidence for antigen processing. J. Exp. Med. 151:314, 1980. K. Molnar-Kimber and J. Sprent. Absence of H-2 restriction in primary and secondary mixed lymphocyte reactions to strong Mls determinants. J. Exp. Med. 151:407, 1980.

159. T CELLS DIRECTED AT A SELF-ANTIGEN - TOLERANCE AND PROLIFERATION TO F

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F is a soluble liver protein, MW 43,000 daltons, which occurs in inbred strains of mice in two allelic variants, 1 and 2. Mice are self-tolerant of F but not at the B cell level since alloimmunization of responder strains leads to the production of autoantibodies that cannot distinguish between the two F types. Chimeras were used to establish that self-tolerance to F is acquired and to measure directly the thresholds for T and B cell self-tolerance. Results confirm that T cells are rendered self-tolerant by far lower doses of antigen than B cells, and are compatible with the hypothesis that experimental autoimmunity can occur when low levels of self antigen are seen by the immune system. T cells can be made tolerant by serum from strains carrying the opposite F type. Cell mixing experiments in cell transfer indicate tolerance is irreversible and no suppresion can be demonstrated.

A T cell proliferation assay for F has been developed. Although F is a potent mitotic inhibitor of both mitogen-induced and antigen-induced proliferation of lymphocytes, this inhibition can be circumvented by pulsing spleen-adherent cells in vivo with F which can then serve as an antigenic stimulus to primed T lymphocytes. This makes possible an analysis of T cell-presenting cell interactions. Results so far indicate that ability to proliferate does not correlate with resonsiveness judged on the basis of antibody production. The phenotype of the proliferating cells will be examined.

160. SPECIFICITY OF ANTIGEN-BINDING RECEPTORS ON T-CELLS

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The summary for this project is under entry no. 134.

161. T CELLS DIRECTED AT VIRAL ANTIGENS - IDIOTYPES

Kees, U., Mitchison, N. A., Imperial Cancer Research Fund, Tumor Immunology Unit, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

T cell blasts anti-Newcastle Disease virus has been used to immunize syngeneic mice against the idiotype of the T cell receptors. The antisera appear to bind specifically to blasts as judged by 125I-anti-immunoglobulin secondary binding.

162. T CELL HYBRIDS WITH ANTIGEN SPECIFICITY

Ruddle, N. H., Yale University, School of Medicine, Dept. of Epidemiology, *333 Cedar St., New Haven, Connecticut,* 06510, United States of America

The purposes of the research are the eventual isolation and analysis of the thymus derived lymphocyte (T cell) receptor for antigen. Continuously propagable T cell lines with specificity for antigen will be developed by means of somatic cell hybridization. Murine T lymphomas will be fused with highly purified populations of murine T cells by means of polyethylene glycol. Primary input parents will include rosette forming cells, effector cells for delayed hypersensitivity, helper cells, cells which suppress the IgE response to ovalbumin, and killer cells. Selection will be accomplished biochemically or with specific antisera. Putative hybrid clones will be analyzed biochemically and functionally. One hybrid which binds sheep erythrocytes has already been obtained. It is expected that hybrids which bind soluble antigen and which express effector functions will also be obtained. This material will be used to analyze the kinetics of T cell antigen binding for receptor isolation.

163. MOLECULAR SPECIFICITY OF CYTOTOXIC T LYMPHOCYTES

Frackelton, A. R., Massachusetts Inst. of Technology, Center for Cancer Research, *77 Massachusetts Ave., Cambridge, Massachusetts,* 02139, United States of America

The purpose of the proposed research is to determine whether anti-vesicular stomatitis virus (VSV) cytotoxic T lymphocytes (CTLs) recognize VSV G glycoprotein or a new antigenic determinant (NAD) formed on the surface of VSV infected mouse cells by the interaction of G with H-2 gene products. The nature of the recognized molecule will be evaluated by comparing CTL activities in cell fractions obtained by affinity chromatography on infected and non-infected fibroblasts. If CTLs recognize G protein, then they should adhere to immobilized, histoincompatible VSV infected fibroblasts but not to immobilized, histoincompatible fibroblasts infected with VSV Ts mutants which do not place G on the fibroblast surface. Specificity of anti-VSV CTL interaction with G will be verified by comparing: (1) the affinity of anti-VSV CTLs for insolubilized, purified G with their affinity for an insoubilized irrelevant glycoprotein; (2) the affinity of anti-lymphocytic choriomeningitis CTLs for insolubilized G with their affinity for an irrelevant glycoprotein. If G is recognized by anti-VSV CTLs, the 3-dimensional rate constants of soluble G binding to CTLs will be measured and converted to 2-dimensional constants, appropriate for the membrane-membrane interactions of CTLs with target cells. The biological implications of these constants will be explored.

164. ANTIGEN-SPECIFIC T-CELL CLONES - GENERATION AND ANALYSIS

Cantor, H. I., Sidney Farber Cancer Center Inc., *44 Binney St., Boston, Massachusetts*, 02115, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

165. SPECIFICITY OF T CELLS IN HUMORAL IMMUNITY (MAMMALS)

Klinman, N. R., University of Pennsylvania, School of Medicine, Dept. of Pathology, *36th & Hamilton Walk, Philadelphia, Pennsylvania*, 19174, United States of America

This is part of a broader project. A summary of this subproject is not available.

166. THE SPECIFICITY REPERTOIRE OF CYTOLYTIC T LYMPHOCYTE

Sherman, L. A., Scripps Clinic & Research Foundation, Dept. of Cellular & Developmental Immunology, *476 Prospect St., La Jolla, California*, 92037, United States of America

This is part of a broader project. A summary of this subproject is not available.

167. INVOLVEMENT OF HLA ANTIGENS IN T CELL ACTIVATION

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OBJECTIVE: To study the involvement of 'self' HLA cell-membrane molecules in activation of T cells against different foreign antigens.

APPROACHES: Using in vitro tests with purified subpopulations of T cells and macrophages, and different antigens as well as antigen expressing target cells.

PROGRESS: We have shown that self HLA-D expressed in macrophages is co-recognized by sensitized T cells when activated by antigens such as PPD and hapten-conjugates. These important questions are now being investigated using antigens of direct relevance to cancer etiology.

168. INTERACTION OF ALLOGENEIC MEMBRANE VESICLES WITH SENSITIZED T-LYMPHOCYTES

Henkart, P. A., Baird, B. A., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Immunology Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

We propose to purify plasma membrane fragments from lymphoid cells and isolate them in the form of resealed right-side-out vesicles containing the water-soluble dye 6-carboxyfluorescein. The interaction of these vesicles with normal and allogeneically immunized lymphocytes will be analyzed using fluorescence microscopy, fluorometry, and fluorescence-activated cell sorting. In the case of allogeneically immunized cells which would be expected to contain killer T lymphocytes, we will determine if the fluorescent dye is specifically released from the vesicles in a manner analogous to that of target cell lysis. Regardless of the outcome of these experiments, we will also study the binding of such membrane vesicles to the whole population of lymphocytes using the cell sorter. This investigation will indicate whether killer T lymphocytes, their precursors, and other functionally demonstrable T lymphocytes (e.g., helper cells) specifically bind allogeneic cell membrane vesicles. If significant binding does occur we will work toward establishing the role of transplantation antigens and Ia antigens in the lymphocyte-vesicle interaction by purifying the antigens, inserting them into carboxyfluorescein filled liposomes, then observing to what extent the previously observed phenomena are repeated.

169. RECRUITMENT OF T CELL IMMUNITY TO ANTIGENS AND TUMORS

Cohen, I. R., Weizmann Inst. of Science, *P.O. Box 26, Rehovot, Israel*

To investigate the cellular events underlying the development of effector T lymphocytes, we separated the intitial induction of sensitization by antigen from subsequent cell movement and differentiation. The aim was to see how the immune system responded to sensitized syngeneic lymphocytes, as distinct from antigen itself. Initiator T lymphocytes (ITL) were sensitized in vitro by culture with allogeneic fibroblasts or with syngeneic macrophages that had been pulsed with specific antigens. Sensitized ITL were collected from the sensitizing cultures and then injected into the foot pads of syngeneic mice. The sensitized ITL migrated to the draining popliteal lymph node (PLN). These ITL did not become effector cells, but trapped other cells from the circulation of the recipient into the PLN. The trapped cells were recruited T lymphocytes (RTL). The population of RTL included precursors of specific cytotoxic effector T lymphocytes, GVH- and transplantation-reactive lymphocytes, helper T lymphocytes and memory lymphocytes. These effector lymphocytes were found to be transiently accumulated in the PLN and relatively depleted from other lymphoid organs. We exploited this depletion to manipulate models of skin and bone marrow transplantation. The information sequentially transmitted between macrophages, ITL and RTL involved the products of genes in the H-2 complex. We succeeded in recruiting effector lymphocytes against antigens shared by syngeneic leukemia cells and leukemogenic viruses.

170. LYMPHOCYTES - GENE EXPRESSION AND ACTIVATION EVENTS

Sell, S., University of California, San Diego Campus, School of Medicine, Dept. of Pathology, *P.O. Box 109, La Jolla, California*, 92093, United States of America

The response of rabbit lymphocytes to different stimuli will be explored further, particularly in regard to a comparison of stimulation of proliferation to stimulation of immunoglobulin synthesis. A simplified assay employing protein A is being developed to measure incorporation of radiolabeled amino acids with immunoglobulins. The T cell requirement for anti-Ig or Con A stimulation of Ig synthesis will be determined and the role of T cells as helper or suppressors of Ig synthesis explored. The expression of immunoglobulin allotypes is being examined in vitro using an inhibition and enhancement of plaque forming cells by anti-allotypic reagents. The effect of allotypic suppression on the expression of other allotypic determinants in vitro is also being examined.

BIBLIOGRAPHIC REFERENCES: Sell, S. The rabbit immune system: Characterization of cell surface markers and functional properties of rabbit lymphocytes. J. Molec. Immunol. 16:1045-1058, 1979.

7. REGULATION OF T CELLS

171. IMMUNOREGULATORY T CELL FUNCTION IN MULTIPLE MYELOMA AND CLL

Ozer, H., Han, T., Henderson, E. S., Cowens, J. W., Roswell Park Memorial Inst., Dept. of Medical Oncology, *666 Elm St., Buffalo, New York*, 14263, United States of America

This project is designed to better delineate the pathology of B cell differentiation and function in two malignancies of immunoglobulin (Ig) synthesizing cells, multiple myeloma (MM) and chronic lymphocytic leukemia (CLL). The specific cellular immunoregulatory mechanisms of T cells, functionally and phenotypically defined T cell subsets, null cells and monocyte/macrophages will be examined with respect to proliferation, differentiation and function of malignant cells and their precursors in comparison with normal differentiation. Although many basic observations in this area have already been made, our approach in this proposal is novel and likely to provide additional new information in a number of areas. Included in these areas of study are T cell regulation of idiotypic Ig production and idiotypic cellular interactions in man; null cell differentiation and function, and null cell suppression of B cell function; T cell subset regulation of autologous mixed lymphocyte responses in myeloma and CLL; and examination of inducer, suppressor and cytotoxic human T cell subset function in these malignancies. In this latter area of investigation we will compare T gamma and T mu subsets with the overlapping but distinct T cell subsets defined by monoclonal antibodies generously provided by Dr. Gideon Goldstein of Ortho Pharmaceutic

Corp., and with inducer and suppressor subsets defined functionally in our own laboratory by sensitivity to the in vitro active metabolites of cyclophosphamide in collaboration with Dr. Michael Colvin of Johns Hopkins University.

172. HUMAN CELL LINE MODELS OF B LYMPHOCYTE INDUCTION AND T LYMPHOCYTE REGULATION

Ralph, P., Sloan Kettering Inst. for Cancer Research, Walker Laboratory, *410 E. 68th St., New York, New York,* 10021, United States of America

This award will support Dr. Peter Ralph of the Sloan Kettering Institute for Cancer Research for a period of six months in a program of research in immunology carried out in cooperation with Dr. Tadamitsu Kishimoto of Osaka University in Japan. Dr. Ralph will spend one month in Japan working with Dr. Kishimoto on studies of the functional interactions between established lines of immunocompetent B cells, T cells, and macrophages. The research will develop these systems as models for biochemical studies on induction and regulation of human immune responses.

173. CELLULAR INTERACTIONS REGULATING THE CYTOLYTIC T LYMPHOCYTE RESPONSE

Burakoff, S. J., Sidney Farber Cancer Center Inc., Dept. of Pathology, *44 Binney St., Boston, Massachusetts,* 02115, United States of America

The summary for this project is under entry no. 77.

174. REGULATORY SIGNALS IN CYTOTOXIC T-CELL DEVELOPMENT

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The summary for this project is under entry no. 81.

175. REGULATORY INFLUENCES OF CELL-MEDIATED IMMUNE RESPONSES

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Previous investigations have described both antigen-specific and antigen-nonspecific T cell-mediated regulatory mechanisms capable of suppressing the in vitro generation of cell-mediated cytotoxicity. Further experiments have been directed at determining the cellular interactions involved in the in vitro generation of suppressor cells, and at analyzing genetic differences in the abilities of different inbred mouse strains to generate suppressor cells.

BIBLIOGRAPHIC REFERENCES: Hodes, R. J. and Hathcock, K.S.: Regulatory mechanisms in cell-mediated immune responses. IV Evidence for the spontaneous generation of suppressor cells in vitro. Transplantation 28: 430-432, 1979.

176. REGULATION OF T CELL MEDIATED ANTI-TUMOR IMMUNITY

Veit, B. C., St. Jude Children's Research Hospital, Division of Immunology, *332 N. Lauderdale St., P.O. Box 318, Memphis, Tennessee,* 38101, United States of America

The purpose of this proposal is to study the mechanisms of interactions between macrophages, lymphocytes and tumor cells in the regulation and development of T cell mediated tumor immunity in an in vitro culture system. Our efforts will be focused on an adherent suppressor cell population, possibly macrophages, which arises in the spleens of tumor inoculated animals and which exerts its effect by inhibiting the formation of cytolytically active T cells. In the event that macrophages are shown to be active in effecting this suppression, methodology will be used to isolate and characterize the specific subpopulation of macrophages responsible for this effect. The latter point becomes especially important when considering various functions ascribed to macrophages (antigen processing or presentation and suppression) since studies of whole populations and macrophages would not provide an accurate account of particular functions but, instead, would indicate the summation of several different functions being performed concomitantly.

In addition to adherent cell suppressive activity, we will also study augmentation of the development of cytotoxic T cells in suppressed spleen cell cultures to which normal thymocytes are added. In this regard, we will consider the possibility that macrophages may be involved in the differentiation of immature thymocytes into cytotoxic effector elements, thereby overriding the suppressive activity.

177. THE CONTROL OF LYMPHOCYTE PROLIFERATION AND DIFFERENTIATION

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The summary for this project is under entry no. 9.

178. CONTROL OF NORMAL AND LEUKEMIC T-CELL PROLIFERATION

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During the past four years it has become apparent that all that is required to maintain the in vitro proliferation of activated T-lymphocytes, is the continual addition of a soluble proliferation trigger. This replication-inducing molecule, termed T-cell growth factor (TCGF), can directly influence the strength and duration of several cellular and humoral immune responses. Purification and iodination procedures have enabled the development of a highly sensitive radiolabeled TCGF binding assay. Such experimentation has revealed that only T-cell populations which proliferate in response to TCGF and absorb biologically active TCGF activity from cultures where it is present possess high affinity cell surface receptors for the growth factor. Resting T-cells lack receptors and without prior ligand stimulation will not proliferate in response to growth factor treatment.

Therefore the degree of T-cell proliferation which serves to direct and mediate the outcome of many immune responses depends not only on the amount of TCGF produced, but presumably on the number of receptor-bearing effector cells generated and the affinity of these cell surface receptors. The research proposed in this application will apply these mechanistic principles of normal T-cell replication to the study of murine leukemia and lymphoma. Using techniques for monitoring TCGF production (TCGF microassay) and use (proliferation, absorption and binding of biologically active and radiolabeled TCGF) we will determine: (1) the TCGF production/use capacities of phenotypically selected subpopulations of normal and immature T-cells and (2) the kinetics and fate of TCGF receptor generation. To determine whether the unchecked replication of malignant T-cells is due to a breakdown in a particular aspect of TCGF-dependent proliferation control, multiple T-cell leukemia and lymphoma samples will be screened for TCGF production and use. Finally, we will attempt to develop a TCGF-specific radioimmonoassay capable of detecting physiologic concentrations (10 to the minus 9th power -10 to the minus 12th power M) of the growth factor. Armed with such an assay it will be possible to probe the in vivo significance of TCGF and determine in a controlled fashion whether TCGF methodologies have the capacity to influence and correct aberrant in vivo immune responses.

179. EXTRINSIC AND INTRINSIC CONTROL OF NORMAL LYMPHOCYTES AND TUMOR PROLIFERATION

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The development of new and more effective ways of employing pyrimidine analogs will be sought by studies of controls on the sources of pyrimidines for tumors compared to normal tissues and the flux of precursors through cytoplasmic compartments as well as the plasma. The action of N-phosphonacetyl-L-aspartic acid (PALA) and pyrazofurin as single agents and in combination with cytosine arabinoside or 5-fluorouracil will be explored. Changes will be studied in the physiological flux (not static concentrations) of pyrimidine nucleosides through the plasma of animals by inhibitors effecting the cellular requirements for these precursors.

Highly purified thymocyte mitogen, Interleukin I, will be studied to define the specific target cells in the thymus and spleen that respond to this monocyte product. Emphasis will be placed on defining functional properties of the mitogenized cell population and the relationship of Interleukin I to the ontogeny of T cell populations.

Cyclosporin A will be used to probe the triggering of T cell replication and also as a potential therapeutic agent in selected T cell malignancies. The sensitivity to Cyclosporin A of a malignant T cell line (BW5147) in culture will permit studies of the mechanism of action using tritiated drug and assays of membrane as well as cytoplasmic events associated with growth. Comparative studies will also be made with clones that have been selected for resistance and with cultures of 'normal' T cells from mice and humans.

180. RESPONSES OF LYMPHOCYTES TO AN ONCOGENIC HERPES VIRUS

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Marek's disease (MD) is a lymphoproliferative disease, caused by a highly contagious oncogenic herpesvirus, Marek's virus. Infection of susceptible chickens with MDV or MD-derived transformed tumor cells (JMV) causes depressed T cell immune responses, as measured in vivo and in vitro. Suppression of T cell immunity may be central to MD pathogenesis. The cellular mechanisms of immunosuppression are being studied. In mixed culture assays, both suppressive and enhancing effects of spleen and thymus cells from MD-infected chickens have been shown. The cells and kinds of interactions which effect these altered responses will be determined. Cell separation procedures, such as ficoll density gradient fractionation, are facilitating these studies. The suppressive or enhancing effects of specific enriched fractions of cells will be tested. Subpopulations of cells separated in the gradient by density are being characterized by cell surface antigens and by immune function. Cultures of lymphocytes, macrophages and tumors from MDV- and JMV-infected chickens will be tested for production of suppressive factors (SF). If found in culture fluids, SF will be studied for its effect on the T cell- and B cell-mediated immune reaction, both in vivo and in vitro. The nature of immunosuppressive effects exerted by MD cells or factors in the various assay systems will be assessed in terms of the severity of disease in the MD-infected donor.

Studies will be undertaken to determine a possible autoimmune component in MD. The proliferation will be measured in mixed cultures with either mitogen-induced or MDV-associated lymphoblasts. Autoreactivity of infected chicken thymus and spleen cells will be studied by GvH reaction. MDV as PBA will be assayed. The cellular basis for vaccine protection against MD oncogenesis will be determined in vivo in transfer systems.

181. T-CELL REGULATION OF ANTITUMOR IMMUNITY

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It is intended to characterize the T-cells that suppress immunity to a murine fibrosarcoma in syngeneic mice. The proposed experiments are based on the background findings (a) that adoptive T-cell-mediated regression of established tumors by intravenous infusion of splenic T-cells from immunized donors can only be achieved with tumors growing in T-cell-deficient recipients, and (b) that this regression can be prevented by intravenous infusion of spleen cells from T-cell-intact, tumor-bearing donors. The suppressor T-cells in these donors will be characterized in terms of their Lyt phenotype, possession of I-J-coded products, sensitivity to ionizing irradiation and cytotoxic drugs, and functional life-span. The kinetics of their generation will be determined in relation to tumor growth and the generation and decay of concomitant immunity. The information obtained will be used to investigate the induction of suppressor cell production by other tumors.

8. OTHER STUDIES ON T CELLS IN TUMOR IMMUNITY

182. LYMPHOCYTES AND MACROPHAGES IN NEOPLASTIC DISEASES OF THE LYMPHOID SYSTEM

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OBJECTIVE: Characterization of tissue lymphocyte functional properties in neoplasia and reactive conditions of central and peripheral lymphoid tissues. Morphologic and functional correlations.

APPROACH: Evaluation of the in vitro proliferative response of lymphocyte suspensions to plant mitogens PHA and ConA.

PROGRESS: A) In myasthenic and non-myasthenic patients the in vitro response to PHA and ConA was determined for thymus lymphocytes from normal, hyperplastic and neoplastic conditions as well as for normal control PBL. Our investigation has revealed the following: 1) Lymphocytes from normal thymus are less responsive to both mitogens than lymphocytes from peripheral blood and abnormal thymus. 2) Lymphocytes from hyperplastic thymus show an increased response only to PHA regardless of the extent of hyperplasia. 3) Lymphocytes from thymoma have an increased response to both mitogens regardless of the histologic pattern of the tumor studied. 4) Thymic lymphocytes from myasthenic patients have a mitogenic response higher than that of thymic lymphocytes from non-myasthenic subjects. 5) In myasthenic patients lymphocytes from neoplastic thymuses show a PHA response higher than that observed for lymphocytes from hyperplastic thymuses. In conclusion, two conditions maximally enhance mitogenic response of thymus lymphocytes: thymomas and myasthenia. B) Lymphocytes from spleens and 28 lymph nodes from Hodgkin's disease (HD) patients were stimulated in vitro with PHA and ConA. The proliferative response of non-involved tissues was found to be twice as high as that of the HD tissues. The percentage number of T lymphocytes, as detected by E rosettes, was found increased in involved as compared to non-involved organs. Furthermore, T cell enrichment of involved populations did not allow recovery of the proliferative response. Depression of proliferative response in involved tissues was also found to parallel the increasing histologic malignancy from lymphocyte predominance to mixed cellularity.

183. B CELL DIFFERENTIATION AND T CELL FUNCTION IN CLL

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CLL is a B cell malignancy with progressive hypogammaglobulinemia. Poor antibody production has been attributed to the malignant B cells failing to generate plasma cells in the immune response. However, it is also possible that a T cell defect may be responsible. A study was therefore made to assess B cell differentiation and T cell function. Separated CLL B cells were stimulated with normal allogeneic T cells and pokeweed nitrogen. Plasma cell production, as identified by fluorescent staining for intracytoplasmic immunoglobulins, has been seen in one of seven patients so far tested. In 3 experiments using allogeneic T cells only as stimulant, there was no evidence of plasma cell production. T cells from patients with CLL, separated by a double rosetting technique, behave normally as responders in mixed lymphocyte culture experiments. CLL B cells generated a normal mixed lymphocyte culture response when used as stimulators of both normal and CLL T cells.

184. ALTERATIONS OF T CELLS IN LYMPHOPROLIFERATIVE DISEASE

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The overall goal of this project is to determine the mechanism of the altered cell surface and function of peripheral blood T lymphocytes in untreated and treated patients with Hodgkin's disease. In particular, we will attempt to characterize a serum factor found in these patients which specifically binds to and alters the function of T lymphocytes from patients with Hodgkin's disease, but not from normals or patients with other cancers. As of the last progress report, we had determined that the specific inhibitory factor in the serum of Hodgkin's patients was altered, after admixture to a potassium bromide gradient, in such a way that the inhibitory activity lost its specificity. The nonspecific inhibitor was found in the LDL fraction of the serum after the KBr gradient separation. On the other hand, separation of the serum of a sucrose gradient showed that the specific inhibitory activity was preserved, but that the activity was not associated with the lipid fraction of the serum. Our most recent results show that the inhibitor obtained from the sucrose gradient can be further purified on the KBr gradient. The material obtained after the second gradient procedure is now associated with the lipid fraction of the serum, and maintains its specificity. The specific inhibitor was further purified by thin layer chromatography, and appears to be a glycolipid. A distinct rapidly migrating band which stains positively for hexose contains the inhibitory activity. This material is found in much greater quantity in Hodgkin's disease as compared to normal serum, as determined by thin layer chromatography. The material runs with an Rf similar to that of galactose or glucose cerebroside. We are presently attempting to elucidate further the sugars and nature of the lipid on the material isolated by thin layer chromatography.

BIBLIOGRAPHIC REFERENCES: Fuks, Z., Strober, S., and Kaplan, H.S. Interaction between serum factors and T lymphocytes in Hodgkin's disease: Use as a diagnostic test. New Engl. J. Med. 295:1273, 1976. Hoppe, R., Fuks, Z., Strober, S., and Kaplan, H.S. Long-term effects of radiation on T and B lymphocytes in the peripheral blood after regional irradiation. Cancer, 40:2071, 1977.

185. CHROMATIN STRUCTURE OF NORMAL AND MALIGNANT T-CELLS

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Normal human thymus-derived lymphocytes (T-cells) may be stimulated to undergo blastic transformation in response to mitogenic stimulation. Early changes in the degree of nonhistone chromosomal protein phosphorylation have been documented to occur following this stimulation. Changes in the phosphorylation of the nonhistone chromosomal proteins have also been demonstrated to occur in association with alteration of chromatin structure and function in these cells stimulated to divide. Given the perturbations in the nonhistone proteins that occur upon stimulation of a quiescent cellular population to divide and given the differences in proliferative potential between normal and malignant lymphocytes, we propose to examine the nature of chromatin structural alterations that occur upon interaction with the phosphorylated nonhistone proteins derived from either normal or malignant T-cells in several phases of the cell cycle. The human malignant T-lymphocytes to be utilized will be cell lines CCRF-CEM and CCRF-HSB-2 derived from two children with lymphoblastic lymphoma. These human cell lines have been shown to be diploid or near diploid, form non-immune rosettes with sheep erythrocytes and lack any Epstein-Barr virus antigen, surface receptors for complement and surface immunoglobulins. The chromatin structural alterations will be examined conformationally, utilizing circular dichroism and ethidium bromide intercalation, as well as in terms of helical stability determined by derivative thermal denaturation. The differential synthesis of phosphorylated nonhistone proteins and their differential binding to nucleohistone will also be examined in the cell populations under consideration.

186. T-LYMPHOCYTE INTERACTION WITH ACCESSORY CELLS IN HUMAN T-LYMPHOCYTE PROLIFERATION

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PROJECT OBJECTIVES: a) To determine which discrete events in the sequence between initial mitogen action and commitment to proliferation of T cells depend on the influence of phagocytic and adherent accessory cells (AC). b) To determine the mechanism of AC influence on T cells. c) To translate the above study (a) to systems using immune stimulants. d) To determine the intrinsic dysfunction of T cells in aging individuals.

PLAN: Specific early metabolic biochemical events are studied in T cells stimulated in the presence and absence of AC.

METHOD: Cell preparations are depleted of AC to the extent that they cannot proliferate. Stimulant is added and glycolysis, various classes of RNA synthesis, K(plus) transmembrane flux, ultrastructural changes and qualitative changes in protein synthesis are studied by standard methods. All studies are done prior to time of commitment to DNA replication.

CLINICAL RELATIONSHIP: Inability of human T cells to respond proliferatively in vivo and conduct normal T cell functions is probably attributable to an intrinsic T cell metabolic dysfunction. By conducting our studies on individuals with documented decrease in T cell responsiveness, we hope to uncover some of the bases of this intrinsic T cell problem, which is ultimately the cause of disease in a significant portion of our patient population.

187. THE USE OF A BIOTIN-AVIDIN SYSTEM TO STUDY INTERACTIONS OF CELL SURFACE GLYCOPROTEINS REQUIRED FOR THE ACTIVATION OF T-LYMPHOCYTES

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No summary has been provided to the Smithsonian Science Information Exchange.

188. A NEW ASSAY FOR T CELL FUNCTION

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The recent rapid advances in cellular immunology have been greatly facilitated by sensitive assays of B cell function, chiefly by enumerating single antibody-forming cells which, in the presence of complement, initiate the lysis of red cell monolayers and form 'plaques' that are readily counted. The study of T cell functions at the single cell level have been hindered by the lack of comparable assays. We have made rabbit and mouse antisera to the products of factors (F) produced by helper and suppressor T cells (HF, and SF) and we have tried over the past two years to use these antisera to develop single cell assays for the T cells that release these factors. Using rabbit anti-HF and rabbit anti-SF, plaque formation has been detected using red cells conjugated with these antisera. The numbers of plaques detected depended upon the source: one plaque was produced per thousand by immunized spleen cells or spleen cell cultures and by one cell per twenty in T cell hybridoma lines that produced either of the two factors. As no plaques were formed using control antisera, the plaques are not due to B cells releasing anti-sheep red cell antibody. The nature of these plaques, which develop much more slowly than B cell plaques, is under analysis to confirm the hypothesis that they are indeed formed by T cells releasing specific factors.

189. INTERACTIONS BETWEEN TUMOR CELLS AND T LYMPHOCYTES

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We propose to study the mechanism by which an IgA myeloma tumor, MOPC-315-EL (BALB/c origin), undergoes a periodic loss, during each transplantation cycle, of the ability to interact with thymus-derived lymphocytes (T). Our approaches will center around the evaluation of the T lymphocyte-tumor cell interaction and the structural change(s) which is responsible for the altered reactivity. The cellular interactions between T cells and the reactive and unreactive MOPC-315-EL tumor cells will be evaluated by 1) T lymphocyte-tumor cell conjugate formation, 2) inhibition of T cell-tumor cell conjugate formation with solubilized, enriched H-2 antigen containing fractions from the reactive and unreactive MOPC-315-EL tumor cells, and 3) by the ability of the solubilized H-2 containing fractions from the reactive and unreactive tumor cells to elicit specific cytotoxic T lymphocytes (CTLs) in culture. Structural changes which might be responsible for the reduced T cell reactivity will be studied by examination of the surface protein, lipid, and carbohydrate composition of the reactive and unreactive MOPC-315-EL tumor cells. A study of the in vivo relationship between the host (BALB/c mouse) and the tumor will be carried out by establishing 1) whether there exists heterogeneity of the MOPC-315-EL tumor cell population, 2) whether a soluble factor is responsible for the induction of the change in reactivity of T cells, and 3) whether this change in reactivity to T cells is an important tumor escape mechanism.

190. EFFECTS OF T CELLS ON THE IMMUNOLOGIC UNRESPONSIVENESS OF B CELLS

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The objective of this project is to investigate the cellular and subcellular mechanisms by which lymphocytes are rendered immunologically unresponsive. Particular emphasis will be placed on B lymphocytes. The presently described mechanisms of unresponsiveness fall into two catagories: intrinsic unresponsiveness and extrinsic inhibition. The proposed research will attempt to define which mechanisms are operative in the unresponsive state established to human IgG (HGG). This project is designed to: a. Clarify the effects of suppressor T cells on the establishment and perpetuation of unresponsiveness to HGG in T and B cells. b. Characterize the components of tolerogen (HGG) which are responsible for the generation of suppressor cells or unresponsiveness devoid of suppression and investigate their mechanism of action. c. Investigate the role of helper T cells in the maintenance and termination of unresponsiveness in B cells. d. Correlate the information obtained on the cellular and subcellular parameters of tolerance to HGG to clinical states of acquired unresponsiveness to foreign antigens and to tolerance to self.

Experimentation will be performed utilizing conventional euthymic mice displaying differing genetically controlled immune responsiveness to HGG, congenitally athymic mice, rabbits, HGG obtained from various diverse sources, and a variety of in vitro cell culture techniques, including polyclonal B cell activation.

191. THE ROLE OF T-CELL FACTOR IN DIFFERENTIATION OF B CELLS

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This is part of a broader project. A summary of this subproject is not available.

192. MODULATION OF T-CELL ACTIVITY AFTER BONE MARROW TRANSPLANTATION

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This is part of a broader project. A summary of this subproject is not available.

193. THYMUS AND DEFECTS OF T CELLS IN IMMUNODEFICIENCY

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This is part of a broader project. A summary of this subproject is not available.

194. FUNCTION AND BIOCHEMISTRY OF T LYMPHOCYTE CLONES

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This is part of a broader project. A summary of this subproject is not available.

195. IMMUNOBIOLOGY OF T LYMPHOCYTES (RATS)

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This is part of a broader project. A summary of this subproject is not available.

B. B LYMPHOCYTES

1. DIFFERENTIATION AND CHARACTERIZATION OF B CELLS

196. STUDIES ON IMPAIRED LYMPHOCYTE FUNCTION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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OBJECTIVE: To explore the mechanism of the differentiation block of B lymphocytes, preventing those from becoming plasma cells, in chronic lymphocytic leukemia, by means of cell culture techniques, and to devise means of overcoming this block. In addition, we wish to use immunological means to increase the number and function of any remaining 'normal' B lymphocyte clones. The eventual object is to find out how to stimulate immunoglobulin (antibody) synthesis in vivo, since low immunoglobulin levels are a common cause of recurrent infections in this disease.

APPROACH: A modification of the Jerne technique in vitro will be employed. This involves culturing B lymphocytes and stimulating them to become plasma cells (antibody-forming cells), which are assayed by plaque formation (hemolysis) of erythrocytes in agar. Various types of stimulants will be tried. Cell separation techniques will be used to test the effect of adding or removing different lymphocyte populations in normal and leukemic cell cultures.

197. B CELL DIFFERENTIATION AND T CELL FUNCTION IN CLL

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The summary for this project is under entry no. 183.

198. IMMUNOBIOLOGY OF EARLY LYMPHOCYTE DEVELOPMENT

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The summary for this project is under entry no. 6.

199. LONG-LIVED LYMPHOCYTES AND SECRETORY BURSAL CELLS

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Our research has revealed the presence of long- and short-lived lymphocytes in the avian thymus, bursa of Fabricius, spleen, and bone marrow. The latter lymphomyeloid tissue possesses the longest lived population of lymphocytes. Whether the long-lived population in the bone marrow is bursa or thymic derived is not known. Therefore, the initial objective of this proposal will be to functionally classify the origin of bone marrow lymphocyte and to assess the lifespan of bone marrow lymphocytes in bursaless chickens. Application of steroids and cyclophosphamide (Cy) in ovo will eliminate B-cells in a large percentage of our birds. While the injection of neonatal chickens with Cy will not eliminate peripheral B-cells, Cy does expand a population of cells in the bursa that morphologically exhibit secretory capabilities and appear to secrete actively. Since Cy and other immunosuppressants are useful in transplantation and in the treatment of human cancer, we need to understand Cy-mediated changes in vertebrates. Therefore, we plan to study the biological activity of the product from the secretory cell.

200. ONCOGENETIC RELATIONSHIP OF CR POSITIVE AND CR NEGATIVE B LYMPHOCYTES

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The aims for the next year's research are threefold:

1. We plan to separate IgD-positive and IgD-negative spleen cells of adult mice with aid of the cell sorter and assess their reactivity in the Mishell-Dutton culture. The expectation is that they will behave like CR-positive and CR-negative cells.

2. We would like to address the issue of differentiation of CR-negative cells. These cells differentiate under the influence of Interleukin 1 to CR-positive IgD-positive cells which are thought to represent the precursors of antibody forming cells. Experiments will be designed to induce differentiation in CR-negative cells, then separate the generated IgD-positive and IgD-negative cells and test separately their ability to form antibody.

3. PC.2 is a new plasma cell antigen defined by monoclonal antibodies. We will investigate the association of this antigen

with cells in transition from resting B lymphocytes (e.g. IgD-positive cells) to antibody forming cells.

BIBLIOGRAPHIC REFERENCES: M.K. Hoffmann and K. Gilbert. Immune functions controlled by an LPS induced macrophage factor (Il-1). Advances in Microbiology. (J. Berry and S. Mergenhagen, eds.) 1979 in press.

201. THE B1-TO-B2 LYMPHOCYTE MATURATION MODEL

Forbes, I. J., Zalewski, P. D., Blake, A. J., Cowled, P. A., Black, R. B., Queen Elizabeth Hospital, Dept. of Medicine, *Woodville, S. Australia 5011, Australia*

OBJECTIVE: B lymphocytes in common CLL have a differentiation block. They are B1 lymphocytes as defined by possession of receptors for mouse erythrocytes, and cannot dfferentiate to B2 cells, which lack this receptor. The objectives are: (1) to define the chemical basis of the membrane change from B1 to B2, (2) to define triggers of this differentiation in vitro and (3) to relate these to factors which may be operating in vivo.

APPROACH: Triggers have been discovered. Of these, action of proteases on the cell surface seems most likely to be a physiological trigger. Cell surface glycoproteins are being analyzed before and after triggering of the B1-to-B2 step. Latex lectin probes are used extensively. The mouse erythrocyte ligand which binds to the lymphocyte receptor is being isolated.

PROGRESS: The mouse erythrocyte lectin is extracted by phenol. The evidence indicates that the B1-to-B2 change triggered by protease is not accompanied by loss or alteration of a range of other surface glycoproteins.

202. NORMAL AND NEOPLASTIC T AND B LYMPHOCYTE MATURATION

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The summary for this project is under entry no. 15.

203. STUDIES ON B CELL MATURATION DEFECT IN NZB MICE

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This is part of a broader project. A summary of this subproject is not available.

204. CELL DIFFERENTIATION STUDIES IN CANCER IMMUNOBIOLOGY

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The summary for this project is under entry no. 5.

205. B CELL LYMPHOMA DIFFERENTIATION

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The research program concerns the analysis of B lymphocyte differentiation through a study of a series of murine lymphomas that may be arrested at various specific points in the B cell maturation pathway. Previous studies have demonstrated that the B lymphocyte system includes several subpopulations of cells, differing in their expression of cell surface antigens and functional properties. In this investigation, B cell heterogeneity will be investigated by the analysis of B lymphoma cell lines. Preliminary studies have suggested that four general groups of B lymphocytes exist: pre-B cells, B cells, Ig-secreting B cells and plasma cells. The goals of the proposed research are: (1) to characterize the surface markers of a series of B cell lymphomas, (2) to analyze the intra-tumor heterogeneity in B lymphoma cell lines, (3) to study the regulation of B cell tumors through the use of anti-idiotypic antisera, (4) to attempt the induction of differentiation in tumors of a given stage with various pharmacological or regulatory agents, and (5) to characterize the nature of surface antigens that are expressed on B cell neoplasms at certain stages of differentiation. Several approaches and techniques will be employed in these studies and particular emphasis will be placed on the use of the flow microfluorometry and cell sorting systems.

206. CHARACTERIZATION OF B CELL PRECURSORS IN REGENERATING BONE MARROW FOLLOWING TERMINATION OF ANTI-LEUKEMIA TREATMENT

Soothill, J., Rapson, N., Paolucci, P., University of London, Inst. of Child Health, Dept. of Immunology, *30 Guilford St., London WC1N 1EH, England, United Kingdom*

The object of the study is to investigate the effect of anti-leukemia treatment on cells of the B lymphocyte series, from the point of view of immunosuppression. Also, since it throws light on the physiology and proliferation control of these cells, which are involved in many cases of leukemia, it may throw light on the leukemic process itself. We wish to characterize the B cell precursors, which are present in raised percentages in regenerating marrow and which may differentiate in short-term culture. Antigens to be tested for will include Ia, ALL and tDT; these are selected because of their association with different maturational stages of lymphocytes.

207. B CELL EXPRESSION OF IMMUNOGLOBULIN V AND C REGIONS

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First, the extent of variable region diversity among murine antibodies to the phosphorylcholine antigen will be examined. Homogeneous antibodies have been produced by hybrid cells from the fusion of normal antibody-secreting cells and myeloma cells. The amino acid sequence of the variable regions of the heavy and light chains will furnish information concerning the nature of antibody diversity.

Second, the heavy chain class of immunoglobulin on the membrane of B cell progenitors will be compared to the class of antibody that is secreted by their clonal progeny. B cells will be stained with fluorescent antisera and separated according to membrane immunoglobulin class using a fluorescence-activated cell sorter. Separated cells will be individually stimulated with antigen in vitro, and the heavy chain class of the secreted antibody made by clonal progeny will be determined by radioimmunoassay. This study may provide insights into the relationship between membrane components of precursor cells and cytoplasmic molecules in differentiated cells.

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208. THE SPECIFICITY REPERTOIRE OF PRECURSORS TO B CELLS

Klinman, N., Scripps Clinic & Research Foundation, Dept. of Cellular & Developmental Immunology, *476 Prospect St., La Jolla, California*, 92037, United States of America

This is part of a broader project. A summary of this subproject is not available.

209. PATTERNS OF DIFFERENTIATION IN VIRUS-TRANSFORMED B CELLS

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This is part of a broader project. A summary of this subproject is not available.

210. ANALYSIS OF B-CELL DIFFERENTIATION

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This is part of a broader project. A summary of this subproject is not available.

2. B CELL SUBPOPULATIONS AND SURFACE COMPONENTS

211. DISTRIBUTION AND FUNCTION OF LYMPHOID SUBPOPULATIONS IN ACUTE LYMPHOBLASTIC LEUKEMIAS AND LYMPHOMAS

Sen, L., Estevez, M. E., Finiasz, M., Diez, R., Guintoli, J., Instituto de Investigaciones Hematologicas, Depto. de Microbiologia, *Pacheco de Melo 3081, 1425 Buenos Aires, Argentina*

The summary for this project is under entry no. 96.

212. BINDING OF BACTERIA TO NORMAL AND LEUKEMIC LYMPHOCYTES

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The summary for this project is under entry no. 100.

213. A SUBPOPULATION OF HUMAN B-CELLS FORMING ROSETTES WITH MOUSE ERYTHROCYTES

Forbes, I. J., Zalewski, P. D., University of Adelaide, Dept. of Medicine, *P.O. Box 498, Adelaide, S. Australia 5001, Australia*

OBJECTIVES: 1. To characterize the population of human B-cells forming rosettes with mouse erythrocytes. 2. To investigate its relationship to chronic lymphocytic leukemia.

APPROACH: The subpopulation of human B lymphocytes (the B1 lymphocytes) forming rosettes with mouse erythrocytes is enumerated by standard techniques. The proportions in different disease states will be investigated. Most of the lymphocytes in chronic lymphocytic leukemia so far investigated have shown this property.

The B1 subpopulation can be isolated by differential centrifugation of rosettes and subsequent lysis of the erythrocytes. The properties of the lymphocytes can then be studied, by measurement of surface markers by fluorescence microscopy and function by incorporation of tritiated thymidine in response to mitogenic stimulation.

The nature of the bond is being studied and compared with the T-cell bond with sheep erythrocytes. Electron microscopy, metabolic inhibitors and fluorescence microscopy are being used.

PROGRESS: The bond is different by a number of criteria from the bond between human T-cells and sheep erythrocytes. There is evidence that the receptor on the mouse erythrocytes binds with a portion of human immunoglobulin on the B-lymphocyte surface. B1 cells are increased greatly in chronic lymphocytic leukemia and in a number of other lymphomas of lymphocytic type.

214. EPSTEIN-BARR VIRUS TRANSFORMATION OF B-LYMPHOCYTE SUBPOPULATIONS SELECTED FOR CLASS OF SURFACE IMMUNOGLOBULIN

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The target cell for Epstein-Barr Virus (EBV) infection has been generally characterized as a B-lymphocyte. The ontogenetic stage(s) at which human B-cells are susceptible to EBV infection is unknown, however. Recent reports by Steinetz et al. suggest that lymphoblastoid cell lines, secreting antibody to specific antigens, may be created by pre-selecting antigen-reactive B-cell subpopulations, then transforming these selected cells with EBV in vitro. These results are promising, but the lack of isotype diversity among the derived cell lines, coupled with previous data in the past decade, make it imperative that the ontogenetic stage(s) of EBV susceptibility among human B-cells should be clarified.

In the present study, the isotype diversity of EBV-susceptible B-cells will be explored, using primary lymphocytes from healthy adults and from cord blood samples. Mononuclear cells from these sources will be submitted to selection procedures to obtain B-cell subpopulations enriched for cell bearing a particular class of surface immunoglobulin -IgG, IgM, IgA, IgD, or IgE. These subpopulations will be infected with EBV in vitro, their transformation efficiency will be measured, and the derived lymphoblastoid cell lines will be characterized for immunoglobulin expression.

215. CHARACTERIZATION AND ISOLATION OF HUMAN LYMPHOCYTE SUBPOPULATIONS

Dickler, H. B., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Immunology Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The long-term goal is to characterize and isolate human lymphocyte subpopulations and evaluate the function of these populations in the immune response of both normal and diseased individuals. Recent work under this project has shown that anti-HL-A alloantisera contain antibodies against antigens which are found primarily on B lymphocytes and which are associated with B lymphocyte Fc receptors. In addition it was found that the Fc receptors of B lymphocytes and UL cells differ both in their avidity for Ig complexes and in the fact that the Fc receptors of UL cells do not appear to be associated with 'Ia-like' antigens. Human peripheral blood mononuclear cells cultured in vitro with the antigen KLH-TNP generated significant numbers (compared to controls without antigen) of plaque-forming cells measured with SRBC coated with staph protein A in the presence of rabbit anti-human Ig, but not when measured with SRBC or SRBC-TNP.

216. IN VITRO STUDIES OF NORMAL AND NEOPLASTIC LYMPHOCYTES

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The summary for this project is under entry no. 108.

217. B-CELL MITOGENS - ACTIVATION MECHANISMS AND EFFECTS ON DIFFERENT B CELL SUBPOPULATIONS

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We are presently attempting to characterize the lymphoid subsets responding to different new B cell mitogens; furthermore, to determine which chemical groups are involved in the actual triggering process of these PBAs, thereby gathering knowledge on the exact structure of the mitogen receptor on cells as well as possible mechanisms of lymphocyte activation and suppression.

The possible influence of regulatory cells (macrophages or suppressor cells) on the thymus-independent immune responses will also be analyzed.

218. BIOPHYSICAL PROBES OF THE LYMPOCYTE MEMBRANE

Owen, C. S., University of Pennsylvania, School of Medicine, Dept. of Pathology, *36th & Hamilton Walk, Philadelphia, Pennsylvania*, 19174, United States of America

The summary for this project is under entry no. 113.

219. RADIATION INJURY IN SUBPOPULATIONS OF LYMPHOCYTES

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The summary for this project is under entry no. 109.

220. MEMBRANE MOLECULES OF B CELLS AND NEOPLASIA

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B cells are selected as subsets defined by the presence (or absence) of the following membrane molecules: Ig, Ia, FcR and lectin responsive glycoproteins, and defined by the density of these glycoproteins on the cell surface. The subsets are stimulated by viral and tumor antigens, whole virus, and tumor cells and by several lectins that bind and may be mitogenic. The response of the B cell subsets to these stimuli is measured in terms of 3HTdr uptake and blastogenesis, Ca(plus plus) fluxes, antibody formation, and cooperation with T cells and macrophages. B cells from young aged and tumor-bearing rats are to be examined by the above procedures.

BIBLIOGRAPHIC REFERENCES: B.A. Woda, J. Yguerabide and J.D. Feldman. 1980. The effect of local anesthetics on the lateral mobility of membrane proteins of lymphocytes. Exp. Cell Res. 126:327. E. Fernandez-Cruz, B.A. Woda, and J.D. Feldman. 1980. Elimination of syngeneic sarcomas in rats by a subset of T Lymphocytes. J. Exp. Med. In Press.

221. INFLUENCE OF B-CELL SUBPOPULATIONS ON GENETIC RESISTANCE TO MAREK'S DISEASES IN CHICKENS

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OBJECTIVE: Determine if the bursa of Fabricius and its subpopulations of B cells influence genetic resistance to Marek's disease.

APPROACH: Several techniques will be used to remove the bursa: Surgical bursectomy (Bx) in ovo, neonatal Bx, injections with cyclophosphamide. B-cell depleted chickens from resistant and susceptible lines will be compared to intact chickens in terms of early and late pathogenetic events: viral internal antigens in thymus, spleen and skin, viral isolation from PBL, thymus and spleen. Influence of Bx on cell-mediated immune (CMI) responses in vitro will be measured by cell-mediated cytotoxicity using T-cells from the spleen and PBL against target tumor cells labeled with 51Cr; and by mitogen and antigen stimulation of PBL measured by the uptake of titrated thymidine. Influence of Bx on in vivo CMI responsiveness will be measured by the time required for rejection of tumor transplants. Reconstitution studies will be used to establish which subpopulation is responsible for the observed effects.

222. MEMBRANE PROTEINS AS MARKERS FOR LYMPHOID TUMORS

Prumers, G. F., Seijen, H. G., Rijksuniversiteit te Groningen, Dept. of Histology, *Oostersingel 69/1, 9713 EZ Groningen, Netherlands*

The summary for this project is under entry no. 102.

223. THE PROGNOSTIC SIGNIFICANCE OF SURFACE MARKERS IN ADULT NON-HODGKIN'S LYMPHOMA IN INDONESIA

Reksodiputro, A. H., Djakaria, M., Santoso, K., Simanjuntak, T., Sudarmo, University of Indonesia, Faculty of Medicine, Dept. of Medicine, *Jln. Yusuf Adiwinata 37, Pusat, Djakarta, Java, Indonesia*

The summary for this project is under entry no. 101.

224. LYMPHOCYTE SURFACE MARKERS AND THROMBOCYTOPHENIA IN BENIGN AND MALIGNANT LYMPHOCYTOSIS

Lassila, O., Kekomaki, R., University of Turku, Dept. of Medical Microbiology, *Kiinamyllynk. 10, SF20500 Turku 52, Finland*

Lymphocyte surface markers are monitored in patients with benign and malignant lymphocytosis. The purpose is to detect the appearance of a monoclonal proliferation of lymphocytes. As a sign of a malignant B-cell proliferation, the following products are measured: paraproteins, immune complexes, autoantibodies. Platelet-associated IgG of patients is quantitated and a possible development of thrombocytopenia is recorded. The prognostic significance of the changes in all of these immunological parameters is evaluated.

225. IMMUNOLOGIC CHARACTERIZATION OF SURFACE MEMBRANE MARKERS IN LYMPHOPROLIFERATIVE NEOPLASMS

Davis, B. K., Shonnard, J. W., Whiteside, T. L., Brubaker, D. B., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, *University Dr. C, Pittsburgh, Pennsylvania*, 15240, United States of America

Our investigative work is presently focused on characterization of heteroantisera directed against lymphocyte populations. Since the activity of many of these antisera (produced against human or rat adult brain, fetal brain, thymocyte or peripheral lymphocyte) is multispecific and relatively refractory to standard absorption techniques, we are currently using more direct, refined techniques of fluoresceination of immunoglobulin fractions and Fab fragments. The majority of these antisera are weakly or non-cytotoxic. However, one reagent produced by mutiple injections of peripheral lymphocytes from a patient with an unusual form of chronic lymphocytic leukemia expressing surface markers typically associated with both T and B lymphocytes had strong cytotoxic activity. This activity was greatest against cells from other patients with more typical CLL with predominantly B cell markers. Only slight cytotoxic activity against normal peripheral lymphocytes or lymph node lymphocytes was detected. Additional target cells are being studied now.

Since the activity of this antiserum was possibly associated with B cell alloantigens, our interest in Ia antigens increased. We have previously demonstrated several specificities that are present on rat B lymphocytes and are associated with antigenic determinants important in mixed lymphocyte responses (MLR). Tissue distribution and functional assays of these antigens parallel those of mouse Ia antigens. Our studies indicated that more than one series of Ia antigens existed in the rat, one associated with MLR and at least one other which was not associated with MLR. We are now using congenic strains of rats and lysostrip and precipitation techniques to further define rat Ia specificities at molecular as well as immunogenetic levels. Hopefully the insight we derive from these animal models will aid in our investigation and interpretation of the patterns of reactivity of leukemic and normal lymphocytes.

226. CHARACTERIZATION OF HUMAN B LYMPHOCYTE DIFFERENTIATION ANTIGENS

Mosier, D. E., Inst. for Cancer Research, Fox Chase Cancer Center, *7701 Burholme Ave., Philadelphia, Pennsylvania*, 19111, United States of America

The research project is aimed at more precise definition of surface membrane markers for B lymphocyte differentiation in the human. A second goal is the identification of membrane components involved in activation of B-cells for antibody synthesis. Finally, a model for the development of B lymphocyte neoplasia will be tested with the markers developed in the initial phase of the study.

The methods involved in the proposal are the preparation and testing of conventional and monoclonal antibodies based on strategies developed in our parallel work on the mouse and the concept that there will be a high degree of molecular homology between murine and human differentiation antigens. Binding studies and correlation of new markers with known B-cell surface receptors will be done using two-color analysis on the dual laser fluorescence activated cell sorter. Membrane components will be characterized using two-dimensional gel electrophoresis. Normal human B-cells, as well as lymphocytes from chronic lymphocytic leukemia and lymphoma patients, will be analyzed for the sequence of appearance of B-cell markers and their presence or absence during pre-neoplastic states. Assuming we identify B-cell receptors for growth-regulatory factors, a hypothesis which states that an initial mutation leads to a loss of regulation of cell proliferation because of the absence of a membrane receptor class can be tested. The role of antigen in the unregulated growth process will also be tested with anti-idiotypic antibodies to characterize clonal expansion.

227. STUDIES OF ANTIGEN RECEPTORS ON HUMAN B LYMPHOCYTES

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The objective of our proposed works is to delineate the structure of IgM class antigen receptor molecules present on human B lymphocytes and to clarify the mechanism of their attachment to lymphocyte membrane.

Human B lymphocytes obtained from chronic lymphocytic leukemia patients and Epstein-Barr virus-transformed lymphoblasts grown by cell culture will be utilized. IgM class antigen receptor molecules will be isolated immuno-specifically from the supernatants of cell lysates. The isolated receptor molecules will be further purified by the combination of various protein chemistry techniques such as gel filtration, ion exchange chromatography, and isoelectric focusing. The purified receptor molecules will be characterized with respect to their molecular weights, chain composition, carbohydrate contents and amino acid sequences by the standard protein chemistry technique. Particular emphasis will be placed on distinguishing various hypothetical mechanisms of attachment of IgM class antigen receptor to lymphocyte membrane.

228. ANTIGEN AND LECTIN RECEPTORS AMONG MEMBRANE GLYCOPROTEIN AND GLYCOLIPID COMPONENTS OF T AND B CELL SUBPOPULATIONS (ABBREV)

Forbes, I. J., Zalewski, P. D., University of Adelaide, Dept. of Medicine, *P.O. Box 498, Adelaide, S. Australia 5001, Australia*

The summary for this project is under entry no. 103.

229. ANALYSIS OF SURFACE STRUCTURES OF HUMAN AND MURINE B LYMPHOCYTES, PLASMA CELLS AND LYMPHOMA CELLS

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OBJECTIVE: We are studying the regulation of B lymphocytes, especially the interactions between macrophages and B lymphocytes, and the participation and role of specific proteinases (partly derived from the complement system) during the induction phase.

APPROACH: In the project described here we want to detect and to characterize various surface molecules (other than Ig molecules and MHC-coded antigens) of human and murine B lymphocytes, and their quantitative and qualitative changes during differentiation. We have selected monoclonal antibodies directed against B lymphocyte- and/or macrophage-specific antigens; we shall analyze their distribution and quantitative patterns, and the formation of neoantigens or antigen complexes during stimulation, and we shall characterize these structures with biochemical methods (labelling, affinity chromatography, two-dimensional PAGE, etc.) and determine their influence in functional assays in vitro and in vivo. The analysis with monoclonal antibodies (in collaboration with other groups in Europe, U.S.A. and Australia) will help us to learn more about the regulatory events on the cell surface of B lymphocytes; it will be extended to include clinical analysis (and probably a study of the influence) of lymphoma cells.

PROGRESS: We have already selected 7 hybridomas (rat plasma and mouse myeloma cells) which recognize mouse B cell (but not T cell) structures other than Ig or H2. We are now characterizing the recognized antigens biochemically, and we are just starting to select (by ELISA and fluorescent techniques) monoclonal antibodies directed against human B lymphocytes and plasma cells. Mice were immunized with B lymphocytes, plasma cells resp. Raji lymphoma cells.

230. RECEPTOR FUNCTION IN LYMPHOCYTE DIFFERENTIATION

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The summary for this project is under entry no. 114.

231. CHARACTERIZATION OF THE LYMPHOCYTE RECEPTOR FOR IGG

Dickler, H. B., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Immunology Branch, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

The goals of this project are to characterize lymphocyte Fc receptors genetically and functionally. Recent findings indicate that the Fc IgG receptors of B lymphocytes interact with a) the lymphocyte cytoskeleton, b) Ia antigens and Lym antigens, c) surface IgM, and d) surface IgD. Each of these interactions is distinct, specific, and non-random. These findings strongly suggest that B lymphocyte Fc IgG receptors are central to B lymphocyte activation and immunoregulation.

BIBLIOGRAPHIC REFERENCES: Klingenstein, R. J., and Dickler, H. B.: The effects of pharmacologic agents on immune complex induced redistribution of B lymphocytes Fc receptors. Scand. J. Immunol. 10: 145-152, 1979. Dickler, H. B., Rosenstreich, D. L., Ahmed, A. and Sachs, D. H.: Genetic linkage between Lym-1, Sas-1 and Mls loci. Immunogenetics 10: 93-96, 1980.

232. GLUCOCORTICOIDS AND LYMPHOCYTE FUNCTION

Ruh, T. S., Ruh, M. F., Nadel, E. M., Bellone, C. C., St. Louis University, School of Medicine, Dept. of Physiology, *1402 S. Grand Blvd., St. Louis, Missouri,* 63104, United States of America

We propose to study the relationship between steroid hormone receptors and function of B-lymphocytes in normal and leukemic (L2C) guinea pigs. Since an early step in the sequence of events which mediates the effect of steroid hormones on their target tissue is believed to be the interaction of the hormones with specific receptor molecules, our approach will be to characterize the glucocorticoid specific receptors of normal guinea pig B-lymphocytes and L2C cells and to relate receptor content and character to function. We will determine the differences or similarities in cytoplasmic and nuclear steroid receptor complexes, the cytoplasmic-to-nuclear-translocation process of the receptor and the nuclear binding characteristics of the receptor of normal B-lymphocytes and the L2C cells. To relate receptor studies to function, the cellular response of B-lymphocytes to mitogen stimulation will be determined in normal and L2C cells, as will the induction of specific proteins which might render the differentiated normal B-lymphocyte resistant to further steroid hormone action. The use of glucocorticoid agonists and antagonists will contribute to the elucidation of the glucocorticoid receptor mechanism in the normal state and in leukemia. These studies are unique since they will make a contribution to our understanding of the normal function of B-lymphocytes and the role glucocorticoids may play in the mediation of the control of cell proliferation. To date very little is known concerning the glucocorticoid receptor and the immune response.

BIBLIOGRAPHIC REFERENCES: Baudendistel, L.J., and Ruh, T.S. Endocrinology 102: 1838, 1978. Baudendistel, L.J., Ruh, M.F., Nadel, E.M., and Ruh, T.S. Acta Endo. 87: 599, 1978.

233. B-LYMPHOCYTE CELL-SURFACE RECEPTORS - STRUCTURE AND FUNCTION OF LYB 3

Davis, M. M., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Allergy & Infectious Diseases, Laboratory of Immunology, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

I wish to characterize the Lyb 3 gene of mice, by enriching for Lyb 3 mRNA, making double-stranded cDNA clones in bacterial plasmids and selecting for the sequences representing Lyb 3. The DNA sequence of relevant clones will be determined and from this the primary amino-acid sequence will be deduced.

234. DIFFERENTIATION AND VIRAL ANTIGENS ON PRIMATE B LYMPHOID CELL LINES

Clark, E. A., University of Washington, Regional Primate Research Center, *c/o Health Sciences Ctr., Seattle, Washington,* 98195, United States of America

The objective of this research is to elucidate the membrane arrangements and surface interactions occurring between lymphotropic herpesvirus structures and B lymphocyte differentiation antigens on primate lymphoblastoid cell lines. A major hypothesis to be tested is whether specific interactions between viral and lymphocyte surface gene products occur which are related to the differentiated state of the cell or whether or not the cell is latently infected on entering the virus lytic cycle. Initial efforts will center on developing and characterizing monoclonal antibodies specific for B lymphocyte differentiation antigens and selected virus-coded membrane antigens. Using these specific probes and methods designed to detect associations between membrane gene products, viral-differentiation antigen arrangements on nonproducer and virus producer cell lines will be determined. Changes in the composite surface phenotype of cells induced to produce viral antigens will be monitored. Family studies and interspecies testing with monoclonal antisera will be undertaken in order to define polymorphic determinants on primate B cells. These markers will be used to examine the relationship between B cell surface phenotype and susceptibility to virus-induced transformation.

235. ROLE OF CELL SURFACE ANTIGENS IN B LYMPHOCYTE DIFFERENTIATION AND MALIGNANCY

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No summary has been provided to the Smithsonian Science Information Exchange.

3. OTHER STUDIES ON B CELLS

236. LYMPHOCYTE REGULATION IN CHRONIC LYMPHOCYTIC LEUKEMIA

Pernis, B. G., Columbia University, College of Physicians & Surgeons, Dept. of Microbiology, *630 W. 168th St., New York, New York*, 10032, United States of America

The research plan is intended to compare the physiological properties of normal human peripheral blood B lymphocytes and of the lymphocytes from patients with B-type chronic lymphocytic leukemia (CLL). The elements to be compared will be the membrane immunoglobulins, with particular attention paid to the ratio of IgM to IgD on single cells, and the ability of the membrane immunoglobulins to interact with other membrane receptors such as the Fc receptor. In addition, attempts will be made to induce the differentiation of CLL lymphocytes in vitro into immunoglobulin secreting cells. This will be attempted employing different T-dependent and T-independent ways of polyclonal activation.

237. HUMAN CELL LINE MODELS OF B LYMPHOCYTE INDUCTION AND T LYMPHOCYTE REGULATION

Ralph, P., Sloan Kettering Inst. for Cancer Research, Walker Laboratory, *410 E. 68th St., New York, New York*, 10021, United States of America

The summary for this project is under entry no. 172.

238. REGULATION OF IMMUNITY BY ANTIBODY AND B CELLS

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B cell proliferation and B cell differentiation have been recognized as distinct components of the humoral immune response. Factors that regulate the immune response may do so by interacting with one or the other of these two B cell functions. Extending our investigation of the regulation of the immune response, we propose to characterize the mechanism of action of immunomodulators - LPS, macrophages, suppressor cells - in these terms. Specifically, we propose to (1) dissect mechanisms of LPS-induced B cell proliferation and differentiation, (2) define effects of LPS on the T cell-dependent production of antibody to SRBC in terms of their relation to proliferation of B cells, (3) determine the role of proliferation signals and differentiation signals in the T cell-independent production of antibody, and (4) characterize suppressor cells in terms of their effects on proliferation and differentiation of B cells.

BIBLIOGRAPHIC REFERENCES: M.K. Hoffman, S. Koenig, R.S. Mittler, H.F. Oettgen, P. Ralph, C. Galanos and U. Hammerling. Control of B cell differentiation by macrophages. J. Immunol. 122:497, 1979.

239. B CELLS DIRECTED AT SELF-ANTIGENS - ANTI-ERYTHROCYTE ANTIBODIES IN NATURAL AND INDUCED AUTOIMMUNITY

Knight, A., Knight, J., Mitchison, N. A., Winchester, G., Imperial Cancer Research Fund, Tumor Immunology Unit, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

We have investigated the cause of spontaneous autoimmune hemolytic anemia in NZB mice. We conclude: (a) that NZB mice do not have a generalized defect in tolerance of self-antigens, as mice with overt auto-immunity were still tolerant of their own liver F antigen; (b) that the specificity of the spontaneously occurring anti-erythrocyte auto-antibodies found in NZB mice is different from that of the auto-antibodies which can be induced in normal and in NZB mice by injecting heterologous erythrocytes, suggesting that the B cell clones responsible for the NZB disease do not exist in normal mice. These findings throw doubt on the validity of the most widely held theories on this subject.

240. REGULATION OF B-LYMPHOCYTE COLONIES

Metcalf, D., Johnson, G., Asano, S., Walter & Eliza Hall Inst. for Medical Research, Cancer Research Unit, *c/o Royal Melbourne Hospital, Melbourne, Victoria 3050, Australia*

Factors controlling the development of B-lymphocyte precursor cells from hemopoietic stem cells will be analysed using a combination of cloning assays in vivo (for multipotential, spleen colony-forming cells, CFU-S) and in vitro (for multipotential colony-forming cells and B-lymphocyte colony-forming cells, BL-CFC).

Spleen colonies generated by fetal liver or adult marrow CFU-S in irradiated CBA and CBA T6T6 recipients will be recloned to analyse their content of BL-CFC and the specificity of these BL-CFC. Modifications of the in vitro cloning technique for multipotential cells will be investigated to develop a cloning system capable of generating BL-CFC in vitro.

Analysis will be made of the mechanism by which purified bacterial cell wall components stimulate the formation of BL-CFC in the spleen and the role played in this process by the serum factor elicited by these bacterial cell wall components. The serum factor will be partially purified and its action in vivo and in vitro on BL-CFC characterized.

Studies will be completed on the response of BL-CFC in mice bearing transplanted B-lymphoid tumors.

241. ROLE OF ACCESSORY CELLS IN B CELL ACTIVATION

Boswell, H. S., Singer, A., Scher, I., Ahmed, A., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Immunology Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

A novel insight into the role of macrophage accessory cells in B cell activation was provided by the observation that the immune response to the thymic independent (T-I) antigen, TNP-Ficoll, was dependent upon the presence and function of splenic adherent accessory cells. The functional spleen adherent cell was identified as a phagocytic macrophagelike cell which bears the I-A gene product. The accessory cell for this T-I TNP-Ficoll response functioned to present antigen to B cells. This identifiable macrophage-B cell interaction was found to be obligately restricted to the Lyb5-positive B cell subset which appears late in normal B cell ontogenetic development and which is absent in hybrid mice which have received the X-linked immune deficiency gene of the CBA/N mouse. The existence of this important cell interaction required for activation of B cells in response to accessory cell dependent antigens such as TNP-Ficoll (T-I) and the thymic dependent (T-D) TNP-KLH provides explanations for the immune response deficit to the T-I and T-D antigens in mice which bear the X-linked CBA/N genetic defect.

BIBLIOGRAPHIC REFERENCES: Boswell, H. S., Sharrow, S. O., and Singer, A.: Role of accessory cells in B cell activation. I. Macrophage presentation of TNP-Ficoll: Evidence for macrophage-B cell interaction. J. Immunol. 124: 989-996, 1980.

242. FUNCTIONAL DEVELOPMENT OF B LYMPHOCYTES

Rittenberg, M. B., Oregon State Higher Education System, University of Oregon Health Sciences Center, School of Medicine, Dept. of Microbiology & Immunology, *3181 S.W. Sam Jackson Park Rd., Portland, Oregon*, 97201, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

243. EVALUATION OF B-CELL FUNCTION IN BONE MARROW TRANSPLANT RECIPIENTS

Pahwa, S., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

244. REGULATION OF B-CELL CLONAL DIVERSITY (MICE)

Kearney, J. F., University of Alabama in Birmingham, School of Medicine, Dept. of Microbiology, *1919 7th Ave. S., Birmingham, Alabama*, 35233, United States of America

This is part of a broader project. A summary of this subproject is not available.

245. DEFECTS OF B LYMPHOCYTES AND IMMUNOREGULATORY CELLS IN PRIMARY IMMUNODEFICIENCY

Pahwa, S., Memorial Hospital for Cancer & Allied Diseases, *1275 York Ave., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

246. CONTROL OF TUMOR GROWTH BY INDUCTION OF B-CELL TOLERANCE (TISSUE CULTURE)

Fuji, H., Roswell Park Memorial Inst., Cancer Cell Center, *666 Elm St., Buffalo, New York*, 14263, United States of America

This is part of a broader project. A summary of this subproject is not available.

247. B-CELL IMMUNOLOGY

Klinman, N. R., University of Pennsylvania, School of Medicine, Dept. of Pathology, *36th & Hamilton Walk, Philadelphia, Pennsylvania*, 19174, United States of America

This is part of a broader project. A summary of this subproject is not available.

C. LYMPHOCYTES (UNSPECIFIED)

1. SUBPOPULATIONS AND SURFACE COMPONENTS

248. IMMUNOCHEMICAL STUDIES OF LYMPHOCYTE MEMBRANE MARKERS IN LYMPHOBLASTIC LEUKEMIAS - CLINICAL IMPLICATIONS

Zappacosta, S., De Felice, M., Del Vecchio, L., Fiorillo, A., Fiore, M., Migliorati, R., Universita degli Studi di Napoli, 2 Fac. di Medicina e Chirurgia, Cattedra di Immunologia, *via Orazio 31, 80100 Naples, Italy*

The project is aimed at the study of some lymphocyte membrane markers in lymphoid cells (peripheral and bone marrow) in the course of acute lymphoblastic leukemia.

Reasons for undertaking such work lie mainly in the availability of a test procedure for DR antigen quantitation, given the role of differentiation antigens currently assumed for such membrane specificities. A radioassay is employed, based on the binding of heterologous antisera to partially purified and radioiodinated DR antigens (obtained from B lymphoblastoid cells or cell lines). The work is done in collaboration with the Pediatrics Department of the same Medical School where protocol No. 7601 for therapy of acute lymphoblastic leukemias of the 'Societa Italiana contro le leucemie' is followed.

Conventional staining procedures and lymphocyte membrane markers are used to classify the disease and to define its different stages.

Another approach involves HLA (A, B, C and DR loci) typing of patients and of their parents in order to search for possible HLA and disease associations and to delineate the pattern of additional reactions often observed in such patients.

249. IDENTIFICATION OF IMMUNOLOGICAL MARKERS IN LEUKEMIC CELLS - CRYOPRESERVATION AND LONG-TERM CULTURES

Gutierrez, R. M., Gonzalez, C. R., Gaminio, G. D., Samano, G. G., Villanueva, T. L., Hospital General SSA, Hematology Service, *Dr. Balmis 148, Mexico 7, D.F., Mexico*

OBJECTIVES: Identifcation of different types of leukemias using specific immune markers. Preparation of specific ALO antisera to permit identification of other leukemias of the same type. Viable conservation by freezing long-term cultures, as a method of obtaining a source of antisera for later immunotherapeutic treatment of the same patient, or a cross-reaction with similar patients.

APPROACH: Peripheral blood samples are taken from patients with leukemia (initially only from patients with acute lymphoblastic leukemia). Using the ficoll-Hypaque technique, separate mononuclei and separate phagocytes from them to obtain only lymphocytes. Trypan blue is needed for viability testing. Rabbits and goats are injected; antiserum absorption; identification of the specific antigen obtained by cytotoxicity.

The study will be repeated with patients in remission. Cryopreservation will be effected in RPMI 1640 and DMSO in liquid nitrogen container. Cultures of lymphoid cells will follow the method of MacCullock and Till.

Techniques and preliminary trials will be standardized.

250. ANALYSIS OF SUPPRESSIVE AND PROLIFERATING LYMPHOCYTES IN HODGKIN'S DISEASE

Wagener, D. J., Haanen, C., Janssen, J., de Pauw, B., Wessels, J. M., University Hospital of St. Radboud, Dept. of Hematology, *Geert Grooteplein 10, 6525 GA Nijmegen, Netherlands*

OBJECTIVE: To study the importance of the lymphocyte subpopulation with strong suppressive properties and the lymphocyte population with high spontaneous proliferative activity in Hodgkin's disease.

APPROACH: Lymphocytes will be isolated from peripheral blood, spleens and lymph nodes. They will be separated into subpopulations using conventional techniques and the cell-sorter.

The lymphocyte subpopulation enriched in suppressor activity and the population with high spontaneous proliferative activity will be characterized, and their significance will be studied.

251. HUMAN MACROPHAGES AND OTHER CELLS IN CARCINOGENESIS

Martin, R. R., Cantrell, E. T., Baylor College of Medicine, School of Medicine, Dept. of Medicine, *1200 Moursund Ave., Houston, Texas*, 77025, United States of America

This proposal is a supplementary request for the project to investigate the induction of aryl hydrocarbon hydroxylase (AHH) in mitogen activated peripheral blood lymphocytes, in pulmonary alveolar macrophages obtained by saline lavage, and in surgically resected lung tissue. The established project is to compare AHH induction in cells and tissues from patients with primary lung cancer, and in patients with other pulmonary diseases. This supplement proposes to obtain pulmonary macrophages and lymphocytes by saline pulmonary lavage from healthy young adult volunteers. These cells will be employed to investigate the effects of cigarette smoking on pulmonary macrophage morphology and function. Specifically, these cells will serve as an approach to establishing the effects of cigarette smoking on the metabolites and the conjugates produced during metabolism of polycyclic aromatic hydrocarbons (such as benzo(alpha)pyrene). These studies will be important in standardizing the approach to investigations involving patient pulmonary macrophages. In addition, preliminary investigations of the cytoskeleton of pulmonary macrophages will be examined, to determine effects which can be attributed to cigarette smoking alone.

BIBLIOGRAPHIC REFERENCES: Martin, R.R., Eknoyan, G., Saenz, C., Hyde, S.E., and Olivero, J. Effects of Renal Failure on Leukotaxis. J. of Med., 10:267-278, 1979.

252. REAGENTS TO CHARACTERIZE CELL SUBPOPULATIONS

Teodorescu, M. C., University of Illinois, Medical Center at Chicago, School of Basic Medical Sciences, Dept. of Microbiology & Immunology, *1853 W. Polk St., Chicago, Illinois*, 60680, United States of America

OBJECTIVE: Determine by in vitro tests the function in immune response of each lymphocyte subpopulation isolated by specific absorption of human lymphocytes to a bacterial monolayer fixed on a glutaraldehyde-fixed gelatin bed. These studies will be limited to functional assays and the diagnostic studies will not be funded.

253. DISTURBANCES OF SUB-POPULATIONS OF LYMPHOCYTES IN PRIMARY IMMUNODEFICIENCY

Gupta, S., Memorial Hospital for Cancer & Allied Diseases, *1275 York Ave., New York, New York*, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

254. CHOLINERGIC RECEPTORS ON LYMPHOCYTES - RELATION TO TRANSFORMATION AND CYTOTOXICITY

Snyder, S. H., Gavish, M., Johns Hopkins University, School of Medicine, Dept. of Pharmacology, *725 N. Wolfe St., Baltimore, Maryland,* 21205, United States of America

A variety of evidence suggests that receptors for acetylcholine of the muscarinic type located on lymphocytes may play a role in their cytotoxicity which in turn is related to the ability of these cells to attack tumors and tissue grafts. Techniques for labeling muscarinic cholinergic receptors with 3H-quinuclidinyl benzilate (QNB) will be applied to lymphocyte populations. Characteristics of lymphocyte receptors will be compared to those of neuronal, glandular and smooth muscle tissues. Changes in the disposition of these receptors will be evaluated as a function of lymphocyte transformation and the induction of cytotoxicity. If, as present evidence suggests, the muscarinic receptors differ to a certain extent in drug specificity from nervous tissue receptors, it may be possible to develop agents which would selectively facilitate host rejection of tumors.

255. CELLULAR ORIGIN OF NON-B LYMPHOCYTES BEARING FC RECEPTORS

Chiao, J. W., Sloan Kettering Inst. for Cancer Research, Laboratory of Immunobiology, *410 E. 68th St., New York, New York,* 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

256. LYMPHOCYTE SURFACE MARKERS

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This is part of a broader project. A summary of this subproject is not available.

257. LYMPHOCYTE SURFACE COMPONENTS AND THEIR RELATIONSHIP TO IMMUNE FUNCTION

Rose, N. R., Wayne State University, School of Medicine, Dept. of Microbiology & Immunology, *1014 Rivard, Detroit, Michigan,* 48207, United States of America

This is part of a broader project. A summary of this subproject is not available.

2. LYMPHOCYTES IN TUMOR IMMUNITY

258. RADIORESISTANCE OF LYMPHOCYTES IN A CASE OF PROLYMPHOCYTIC LEUKEMIA

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Prolymphocytic leukemia is a rare variant of chronic lymphocytic leukemia (CLL) distinguished from the classical disorder by the morphology of the lymphoid cells and by characteristic clinical symptomatology (Galton et al., British Journal of Haematology, 1974, 27, 7). In the present case, the lymphocytes were found to show several additional peculiar features (Publication No. 207). The majority had markers of both 'T' and 'B' lymphocytes, but in common with CLL=lymphocytes the majority were colchicine ultrasensitive. Unlike CLL-lymphocytes or normal lymphocytes they were highly radioresistant in vitro.

Exposure of the patients' peripheral blood lymphocytes to the highest dose of radiation tested (500 R) elicited only 15 percent death during a subsequent two-day period. Mortalities recorded previously for normal and CLL lymphocytes were higher and particularly so in CLL where they measured 40-87 percent.

Although the patient radiation response - as expressed by a plot of the logarithm of lymphocyte survival against radiation dose (0-500 R) -could be drawn as a straight line, it was considered impermissible to extrapolate it to doses above 500 R even if the remaining 85 percent of the population were assumed to be equally radiosensitive. Theoretical considerations have shown that the radiation response of a homogeneously radiosensitive population that remains incompletely lethally irradiated up to the highest dose tested will be non-linear at any time of analysis prior to death of all the lethally irradiated cells. Moreover, according to a mathematical equation that predicts the shape of the radiation response where death rate is governed by first order kinetics (ICRF Scientific Report 1976 - report 113) the response at the most lethally ineffective doses will appear linear. Extrapolation of the experimental findings in accordance with this equation gave 2,500 R as the estimate of radiosensitivity (i.e, the dose permitting 37 percent survival). This value denotes marked radioresistance when compared with values all less than 400 R found for CLL-lymphocytes, normal T lymphocytes or normal B lymphocytes.

259. MONOCYTE-LYMPHOCYTE INTERACTIONS IN MYCOSIS FUNGOIDES

Weston, W. L., University of Colorado, Health Sciences Center, School of Medicine, Dept. of Dermatology, *4200 E. 9th Ave., Denver, Colorado,* 80262, United States of America

The objective of this project is to study monocyte-lymphocyte interactions in patients with mycosis fungoides to better understand the pathogenesis of this disease and as a model for cellular cooperation in other malignant diseases and in normal individuals. Cellular interaction will be measured in mixed leukocyte reactions, mitogen-induced antibody production and blastogenic responses, co-stimulator assays, and monocyte and lymphocyte chemotaxis experiments. Cells will be fractionated by standard density and adherence techniques and also by counterflow centrifuge separation (elutriation) to carefully distinguish contributions by monocytes and lymphocytes. Tissue transudates from MF lesions will be tested in vitro for effects on cellular stimulation and on cellular movement.

260. LYMPHOCYTE FUNCTION IN PATIENTS WITH MYCOSIS FUNGOIDES

Van Scott, E. J., Temple University, School of Medicine, Dept. of Dermatology, *3322 Broad St., Philadelphia, Pennsylvania,* 19122, United States of America

This is part of a broader project. A summary of this subproject is not available.

261. AUTOREACTIVE CELLS IN TUMOR PATIENTS

Klein, E., Vanky, F., Masucci, G., Argov, S., Patarroyo, M., Karolinska Institutet, Dept. of Tumor Biology, *Solnavagen 1, S10401 Stockholm 60, Sweden*

Patients will be investigated with regard to their immunological reactivity against their own tumors (lung tumors, osteosarcoma, astrocytomas) using two in vitro assays: ATS-autologous tumor stimulation which measures blastogenesis of lymphocytes exposed to the biopsy cells and ALC-autologous lymphocyte cytotoxicity exerted towards biopsy cells. In our past research a certain proportion of patients have exhibited such reactivities. In a further step the optimal conditions for generating cytotoxic cells in vitro by cocultivation with biopsy cells will be explored. It is possible that such in vitro generated reactive cells can be exploited in the future for immunotherapy.

Tumor patients with the same and different histopathology will be tested for cross-reactivity in the ATS and ALC assays. Preliminary experiments indicated that when biopsy preparations highly enriched for tumor cells are used, reactivity due to alloantigens does not occur.

A prerequisite for the above delineated studies is the separation of biopsy into tumor and reactive cell components. The methodology has been worked out in our laboratory. This also allows the study of the nature of infiltration and the function of infiltrating cells in vitro. Thus information about in situ tumor immunity will also be obtained.

262. LYMPHOCYTE FUNCTION IN BREAST CANCER

Harrison, R., Knight, S., Farrant, J., Clinical Research Centre, Oncology Unit, *Watford Rd., Harrow, Middlesex HA1 3UJ, United Kingdom*

OBJECTIVE: To investigate whether the study of two in vitro factors, (1) cell concentration and (2) harvest time, clarifies the relationship between the observed pattern of lymphocyte function and the clinical states of these.

APPROACH: Lymphocyte transformation can be induced by specific or nonspecific stimuli. This transformation may be measured by estimation of DNA synthesis. Lymphocyte transformation has been measured in patients with malignant disease but the correlation with the clinical stage of the disease has been variable. It has been established that a range of cell concentrations is important in assessing lymphocyte function (Knight S. and Farrant J., 1978, Journal of Immunological Methods 22 63-71). With the development of a simple harvester for 20 microlites of Terasaki cultures (O'Brien et al., 1979, Journal of immunological Methods, in press) and the relization that different harvest times are also required (Farrant J. and Knight S., in preparation) it is proposed that a re-examination of the relationship between lymphocyte function and disease is undertaken utilizing these new techniques. All new patients presenting with carcinoma of the breast and those presenting with recurrent disease will be considered for inclusion in the study. Initially 1-2 patients per week will be studied as dictated by available laboratory facilities.

PROGRESS: Patient entry to this study has now been limited to post-menopausal women to make the subsequent analysis of a single biological group possible. Control studies confirm the reproducibility of the method and are continuing to look at any natural variability.

263. RESPONSE OF LEUKOCYTES TO HUMAN TUMOR CELLS

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Human tumor cells currently carried in culture, as well as cells obtained from patients at surgery, will be examined for their capacity to stimulate allogeneic lymphocytes. Tumor cells will be classified as stimulatory, (S positive), or non-stimulatory, (S negative), depending upon whether they induce a response against themselves in allogeneic mixed leukocyte cultures. We will attempt to induce stimulation by S negative cells through the addition to cultures of a factor isolated from the supernatant of lectin stimulated lymphocytes-monocyte populations (co-stimulator). This has already been done for the S negative colon tumor, HT29.

Next, the functions of subclasses within the responding cell population will be examined. Counterflow centrifugation will be used to isolate pure populations of small lymphocytes, large monocytes and an intermediate population consisting of a mixture of large lymphocytes and small monocytes. The effects on the cytotoxic response of varying the ratio of the relative numbers of small lymphocytes and large monocytes will be studied, along with the perturbations created by the intermediate fraction. We have already established that a stronger cytotoxic reaction is mounted against the S positive Lymphoma, RPMI 8866, when the intermediate fraction is removed from the responding cell population, perhaps indicating the presence of suppressor cells.

If S negative tumors removed at surgery can be converted to S positive by costimulator, the leukocytes of the tumor bearers will be examined for their ability to produce that factor. We have recently demonstrated that a costimulator preparation made from the cells of a normal donor appeared to contain a suppressor substance which prevails over the stimulatory factor at high but not at low concentrations. Changes in the properties of costimulator will therefore be examined, for both normal donors and cancer patients, when the cell populations from which it is prepared are selectively depleted of their various components.

264. MODULATION OF LYMPHOCYTE CYTOLYSIS OF TUMOR CELLS

Fuson, E. W., University of Tennessee, Center for the Health Sciences, Memorial Research Center & Hospital, Dept. of Medical Biology, *1924 Alcoa Hwy., Knoxville, Tennessee*, 37920, United States of America

We have demonstrated that immobilized IgG aggregate or IgG-erythrocyte complexes, lacking target cell specificity, may induce lysis of erythrocytes by normal human peripheral blood lymphocytes (PBL). The reaction induced by immobilized IgG aggregate can be distinguished from that induced by IgG-erythrocyte complexes by differences in susceptible target cells, kinetics and suppression by other cells. Furthermore, both reactions are easily distinguished from antibody dependent cell-mediated cytotoxicity (ADCC) and natural or spontaneous cell-mediated cytotoxicity (NCMC). During the next budget period we will attempt to further define these reactions. Comparisons will be made between ADCC, NCMC, immobilized aggregate and IgG complex induced cytolysis. Special emphasis will be placed on the difference in target cells lysed in IgG aggregate and IgG-erythrocyte complex induced cytolysis (IgG erythrocyte induced lysis is specific for SRBC). Effector cells and suppressor cell characteristics will also be determined.

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265. FUNCTIONAL DIVERSITY OF DIFFERENTIATING LYMPHOCYTES

Miller, H. C., Michigan State University, School of Human Medicine, Dept. of Microbiology & Public Health, *Soil Science Bldg., East Lansing, Michigan*, 48824, United States of America

In order to immunologically correct aberrant regulation of lymphopoiesis which leads to cancers such as lymphomas, myelomas and leukemias, an understanding of normal regulatory events is needed. This project is designed to investigate changes which take place as stem cells differentiate into clones of functional lymphoid cell populations. The experimental approaches will make use of an in vitro system to elucidate the steps of maturation and the regulatory mechanisms which direct biophysically purified precursor cells in their development into functional helper, effector, memory and suppressor lymphocytes. Stem cell or precursor cell surface changes will be followed using membrane probes to correlate molecular modifications that arise during differentiation with lymphoid acquisition of functional capacity. Selective maturation and induction of functional lymphocyte populations in vitro could then be used to repair immunodeficiencies or to provide appropriate numbers of antigen-specific lymphocytes that are capable of restricting growth of neoplastic tissue in situ. Memory cells could be induced which would supply backup numbers of hyperactive effector or surveillant lymphocytes.

266. CONTROL OF IMMUNE AND MALIGNANT LYMPHOCYTE GROWTH

Mendelsohn, J., Caviles, A. P., Hudig, D., University of California, San Diego Campus, School of Medicine, Dept. of Medicine, *P.O. Box 109, La Jolla, California*, 92093, United States of America

We will explore the effects upon human lymphocyte proliferation of monocytes and other cellular and humoral elements in cultures as well as regulatory effects of hormones and chemotherapeutic agents. Stimulation and control of DNA synthesis and cell division will be studied in cultures of lymphocytes obtained from normal donors, patients with immunological diseases and patients with lymphocytic malignancies. Accurate quantitation of the number of responding cells and of cell cycle kinetics will be carried out with lymphocytes exposed to nonspecific mitogens and antigens. The role of monocytes in lymphocyte-mediated 'natural' or 'spontaneous' cytotoxicity against tumor cells will also be investigated.

BIBLIOGRAPHIC REFERENCES: Dorian, R., J.L. Bernheim and J. Mendelsohn. Quantification of entry into and exit from the cell cycle in human lymphocyte cultures. Cell Tissue Kinet. 11:33-44, 1978. Shiftan, T.A., A.P. Caviles, Jr. and J. Mendelsohn. Spontaneous lymphocyte proliferation and depressed cellular immunity in Hodgkin's disease. Clin. Exp. Immunol. 32:144-152, 1978.

267. CONTROL OF GROWTH IN QUIESCENT HUMAN CELLS

Norin, A. J., Bloom, B. G., Emeson, E. E., Montefiore Hospital & Medical Center, Dept. of Microbiology & Immunology/Surgery, *111 E. 210th St., Bronx, New York*, 10467, United States of America

The long-term goal of this project is to understand the regulatory mechanisms which prevent cells from proliferating though they have the capacity to do so. These mechanisms are extremely important to the organism since their failure would result in catastrophic, uncontrolled growth. Therefore, elucidation of growth control mechanisms is one of the prime objectives of cancer research. The reaction of a variety of substances, mitogens, with the lymphocyte membrane can alter metabolism and induce proliferation in this normally quiescent cell. An understanding of the essential processes which cause activation may allow one to determine how cells maintain a quiescent state. One problem in interpreting biochemical data from mitogen activation experiments is the heterogeneity of lymphocyte cultures. A sizable proportion of cells may not respond to stimulant and responsive cells are extremely asynchronous with respect to the time at which they synthesize DNA. The immediate objectives of this project are 1) to study further the behavior of lymphocytes in culture using DNA density transfer technology and 2) to develop a method of isolating homogeneous subpopulations of quiescent human lymphocytes that can be activated in a relatively synchronous manner. The strategy of the latter is based, in part, on the observation of my colleagues and me that mitogen activated lyphocytes divide and then become quiescent unless stimulant is present. Thus, activation can be studied in the quiescent progeny cells since, like their progenitors, reactivation also requires reaction of mitogen with the cell membrane. A more homogeneous population of mitogen responsive lymphocytes may be obtained using a method I propose to develop based on my previously published work. Activated lymphocytes committed to synthesize DNA remain attached to sepharose beads (which have concanavalin A covalently bound) even in the presence of the binding competitor methyl-a-d-mannoside (MAM). The MAM can dissociate unactivated or uncommitted cells from the beads. Physical separation of the dissociatable populations from the nondissociatable population followed by removal of attached cells by mechanical means should be helpful in isolating more homogeneous lymphocyte cultures for further study.

268. IN VITRO GENERATION OF CYTOTOXIC LYMPHOCYTES

Rinehart, J. J., Ohio State University, Columbus Campus, Office of the Vice-President for Medical Affairs, *190 N. Oval Dr., Columbus, Ohio*, 43210, United States of America

This is part of a broader project. A summary of this subproject is not available.

269. STUDIES ON TUMOR-RESISTANT LYMPHOCYTES OF THE ATHYMIC MOUSE

Kawamura, A., Hamajima, K., Suzuki, K., University of Tokyo, Inst. of Medical Science, Dept. of Immunology, *P.O. Takanawa, Tokyo, Japan*

Histopathologically, lymphocytic reaction to proliferation and metastasis of the tumor has been observed in athymic mice heterotransplanted with tumor cells. This immunologically resistant response of the athymic mouse has been analyzed in the ADCC system (in vitro and in vivo). The experimental system demonstrates that a certain subset of lymphocytes (in particular, m phi) of tumor-bearing athymic mouse plays an important role in the tumor-resistant mechanism. On the basis of this result, the adjustment of effector cells is now examined using various methods in regard to separation procedures of m phi (enrichment and depletion).

This active immunoresistant mechanism provides a clue to immunosurveillance of human malignancy.

270. IMMUNOSUPPRESSANTS AND LYMPHOCYTE FUNCTION

Winkelstein, A., University of Pittsburgh, Montefiore Hospital, Dept. of Medicine, *3459 5th Ave., Pittsburgh, Pennsylvania*, 15213, United States of America

The proposed studies are primarily directed at defining the effects of immunosuppressive drugs on lymphocyte numbers and their functional activities. Results are to be correlated with changes in parameters of immunity. One aspect of the investigation concerns an assessment of the activity of various cytotoxic drugs on murine NK cell function and B cell colony formation. In addition, these drugs will be tested for the comparative effects on antibody responses to thymic dependent and independent antigens. Correlative studies using human T cell colony assay will be undertaken in order to evaluate patients with suspected T cell defects. These include those with connective tissue diseases and individuals receiving cytotoxic drug therapy. Another area of study will be directed at the potential therapeutic activities of PGE1 in murine models of SLE. We have shown that this agent markedly prolongs survival in the NZB/W and MRL/l strains and that these beneficial effects are associated with distinctive lymphoid changes. However, therapeutic activity of this agent is not due to generalized immunosuppression; rather, it appears to selectively inhibit certain immune responses. The latter is manifested by reduction in circulating immune complexes. The relationships between lymphoid changes, parameters of immunity and increased survival will be further evaluated.

BIBLIOGRAPHIC REFERENCES: Winkelstein, A., and Kelley, V.E.: The pharmacologic effects of PGE1 on murine lymphocytes. Blood 55:437-444, 1980. Winkelstein, A. Principles in the use of immunosuppressive agents. In Primer on the Rheumatic Diseases (G.P. Rodnan ed.) 8th edition, 1980 (in press).

271. THE ROLE OF RABBIT LYMPHOID CELLS IN TUMOR IMMUNITY

Sell, S., University of California, San Diego Campus, School of Medicine, Dept. of Pathology, *P.O. Box 109, La Jolla, California*, 92093, United States of America

The immune response to Shope fibroma virus induced tumor in adult rabbits which exhibit regression is being compared to that of neonatal rabbits which experience progressive tumor growth. The kinetics of development of cytotoxic lymphocytes in newborn rabbits will be determined. Other parameters of immune reactivity both specific and non-specific will be tested to determine if there is a significant immune defect in neonatal rabbits. Finally, attempts will be made to modify the course of tumor growth in neonatal rabbits by transfer of cells and products from adult 'regressor' donors.

272. EFFECTOR LYMPHOCYTE - TUMOR CELL INTERACTION

Lindquist, R. R., University of Connecticut, Health Center, School of Medicine, Dept. of Pathology, *Farmington Ave., Farmington, Connecticut*, 06032, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

273. LYMPHOCYTE RESPONSE IN VITRO TO WHEAT GLUTEN ANTIGEN IN CELIAC DISEASE

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This is part of a broader project. A summary of this subproject is not available.

274. LYMPHOCYTE RESPONSES TO ALLOGENEIC CELLS (MICE, RATS)

Elkins, W. L., University of Pennsylvania, School of Medicine, Dept. of Pathology, *36th & Hamilton Walk, Philadelphia, Pennsylvania*, 19174, United States of America

This is part of a broader project. A summary of this subproject is not available.

3. OTHER STUDIES ON LYMPHOCYTES

275. STUDY OF PERIPHERAL BLOOD AND BONE MARROW LYMPHOCYTES IN MULTIPLE MYELOMA

Di Padova, F. E., Bianchi, G., Morandi, E., Baldini, L., Chatenoud, L., Palazzi, P., Mozzana, R., Universita degli Studi di Milano, Dept. of Clinical Immunology, *via F. Sforza, 35, 20122 Milan, Italy*

OBJECTIVE: The aim of this study is to define the morphologic and functional characteristics of peripheral blood lymphocytes and of bone marrow lymphocytes and plasma cells in multiple myeloma and related conditions.

APPROACH: 1) We are evaluating the morphologic and functional differences between peripheral blood lymphocytes of patients with multiple myeloma and of controls. We are using the following methods: early E rosette test; E rosette test; tests for detection of surface immunoglobulin bearing lymphocytes by direct immunofluorescence; blastogenic response to PWM and to PHA. We have already observed interesting differences between patients and controls and between different classes and types of multiple myeloma. 2) We are now introducing other tests: a) to define the kinetics of appearance of cytoplasmic and secretory immunoglobulins in peripheral blood lymphocytes cultured with PWM; b) to study other surface markers on peripheral blood lymphocytes and on bone marrow cells (T mu; T gamma); c) to characterize the secretory products, mainly immunoglobulins, of peripheral blood lymphocytes and of bone marrow cells (lymphocytes and plasma cells) in culture; d) to define the role of serum and cellular factors in differentiation of peripheral blood and bone marrow cells.

PROGRESS: We are confirming in a larger population of patients the results obtained and we are introducing the tests reported on under (2).

276. PROTEIN KINASES IN NORMAL AND LEUKEMIC LYMPHOCYTES

Martelo, O. J., University of Cincinnati, School of Medicine, Dept. of Internal Medicine, *Eden & Bethesda Aves., Cincinnati, Ohio,* 45221, United States of America

The overall goal of this research project is to determine the role of cyclic AMP and cyclic GMP in protein synthesis and RNA synthesis in normal and leukemic lymphocytes.

Since cyclic nucleotides function predominately in cells by activation of protein kinases, this project has also studied the binding of cyclic nucleotides to the regulatory subunit of protein kinases in homogenates of lymphocytes. The phosphorylation of endogenous lymphocyte proteins in the presence of gamma-32-ATP using a two-dimensional isoelectric focusing technique was determined.

In comparing normal lymphocytes with chronic lymphocytic leukemia lymphocytes, we have not noted any differences in the properties of the protein kinases or endogenous phosphorylation patterns. However, differences have been noted in comparison of patterns of phosphorylation of endogenous proteins in lymphoblasts with normal controls.

Examination of the two-dimensional isoelectric focusing autoradiograms of lymphoblasts demonstrates an absence of phosphorylated protein of approximately 200,000 molecular weight and having an isoelectric point of approximately 5.5.

The plan is to pursue the significance of these interesting findings.

277. BIOCHEMISTRY OF NORMAL AND LEUKEMIC LYMPHOCYTES

Abell, C. W., Monahan, T. M., University of Texas, Medical Branch at Galveston, School of Medicine, Dept. of Human Biological Chemistry & Genetics, *300 University Blvd., Galveston, Texas,* 77550, United States of America

The overall objectives of this study are to contribute to a better understanding of the mechanism of mitogenic activation of lymphocytes, and to compare membrane and soluble components of lymphocytes from healthy individuals and patients with chronic lymphocytic leukemia. We will examine cell-to-cell interactions and biochemical events that occur during activation of lymphocytes with sodium periodate (NaIO4) and galactose oxidase. The role played by different cells (stimulator cells, responder cells, and adherent cells) in oxidative activation of lymphocytes will be investigated. In attempts to detect specific surface components involved in the activation, membrane preparations will be resolved by lectin-Sepharose chromatography and by two-dimensional gel electrophoresis. To study soluble factors produced soon after activation, adherent cells and either stimulator or responder cells are physically separated by layers of agarose. The purification of soluble factor from adherent cells and a lymphocyte growth factor from stimulator cells will be pursued. Biochemical differences between lymphocytes obtained from healthy donors and those from patients with chronic lymphocytic leukemia will be compared on the basis of cell-to-cell interaction, cell surface components involved in activation, and soluble factors.

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278. IMMUNOBIOLOGICAL STUDIES WITH CULTURED HUMAN LYMPHOCYTES

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This is part of a broader project. A summary of this subproject is not available.

279. GENETIC CONTROL OF HUMAN MIXED LYMPHOCYTE CULTURE REACTIONS

Dupont, B. O., Sloan Kettering Inst. for Cancer Research, Research Histocompatibility Testing Laboratory, *410 E. 68th St., New York, New York,* 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

280. CHARACTERIZATION OF THE IMMUNE LYMPHOCYTES IN AGING NORMAL AND TUMOR-BEARING ANIMALS

Veronese, F., Kelloff, G. J., Kende, M., Stephenson, J. R., Witz, I., Nicolin, A., Dinowitz, M., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Viral Immunology Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

This project has the long-range goal of prevention by immunological means of naturally occurring or chemically induced tumors in animal model systems that have viral, immunologic and epidemiologic similarities to human tumors. To this end, the project has defined the spontaneous tumor and endogenous type C virus expression in colonies of untreated aging mice and has measured the host's natural and induced immune response to same. Certain immunization regimens have been found by this project and other investigators to induce an immune response in the host that is effective in preventing experimentally induced tumors and type C virus expressions. Therefore, the major emphasis of the project now is to specifically define the effector immune responses that occur and are responsible for the successful prevention of tumors, whether naturally occurring or induced. To achieve this, additional techniques specifically involving lymphocyte separation and characterization are being established. An equivalent emphasis is being placed on the nature and specificity of the target antigens that these immune responses are directed against, especially with regard to the gene products of the type C virus including the more recently defined transforming proteins.

281. REGULATION OF EARLY EVENTS IN LYMPHOID DIFFERENTIATION

Phillips, R. A., Ontario Cancer Inst., Division of Biological Research, *500 Sherbourne St., Toronto, Ontario,* M4X 1K9, Canada

OBJECTIVE: To establish an in vitro system in which there is continuous production of functional lymphoid cells from multipotent stem cells.

APPROACH: Long-term cultures of murine bone marrow cells are established following the method of Dexter. At weekly intervals, several tests of lymphoid function are made: 1) Cells are injected into irradiated mice and tested for their ability to reconstitute lymphoid function; the donor and host cells can be distinguished by unique chromosome markers. 2) Cells are incubated with lipopolysaccharide, a B cell mitogen; antibody-producing cells are measured between days 4 and 9. 3) Cells are incubated in semisolid medium with specific inducers of T lymphocyte colonies.

PROGRESS: 1) The cultured bone marrow cells have the ability to produce functional B and T lymphocytes in irradiated recipients; this regenerating potential can be maintained for at least 7 weeks in vitro. 2) Functional, LPS-reactive B lymphocytes have never been detected in long-term cultures. 3) T lymphocyte colony-forming cells are detectable in cultures for up to 17 weeks. The cells in the colony bear the Thy 1 surface antigen, but we do not yet know if the cells in the colony have any functions commonly associated with T lymphocytes.

282. LYMPHOCYTE FACTORS IN NORMAL AND LEUKEMIC MICE

Di Sabato, G., Vanderbilt University, School of Arts & Sciences, Dept. of Molecular Biology, *21st Ave. S., Nashville, Tennessee,* 37232, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

283. IDENTIFICATION, ISOLATION AND ANALYSIS OF LYMPHOCYTES

Cooper, M. D., University of Alabama in Birmingham, School of Medicine, Dept. of Pediatrics/Microbiology, *1919 7th Ave. S., Birmingham, Alabama*, 35233, United States of America

This is part of a broader project. A summary of this subproject is not available.

D. K AND NK CELLS

1. CHARACTERIZATION OF K AND NK CELLS

284. RELATIONSHIP BETWEEN SURFACE MARKERS AND CYTOCHEMICAL STAINS IN ALL

Donoso, J., Taboada, H., Grebe, G., Universidad Catolica de Chile, Depto. de Pediatria, *Casilla de Correo #89, Puente Alto, Chile*

OBJECTIVE: To determine the relationship between these two methods of classifying acute lymphatic leukemia in children, with emphasis on determining the relationship between subpopulations of T leukemias and acid phosphatase stain.

METHOD: Upon diagnosis, studies are performed on membrane receptors using the rosette formation test (T lymphocytes) and the synthesis of immunoglobulins (B lymphocytes), with which the leukemia is classifed into T-B-null.

Then studies are performed with cytochemical stains (PAS acid phosphatase), and clinical studies are performed (thoracic X-ray, etc.).

The evolution of the child is followed to observe the behavior of the various subgroups, with special focus on the 'null' acid phosphatase positive leukemias.

PROGRESS: This study has been underway for one year, during which time 35 children have been studied. It has been determined that 90% of the T leukemias are acid phosphatase positive. The significance of the other subgroups remains to be seen.

285. LYMPHOCYTE DIFFERENTIATION IN CHILDHOOD LEUKEMIA

Kaplan, J., Children's Hospital of Michigan, *3901 Beaubien St., Detroit, Michigan*, 48201, United States of America

This project seeks to investigate the nature of the cells of origin of acute childhood leukemias and the factors which govern their differentiation and proliferation. We plan to increasingly focus on the possibility that natural killer (NK) cells are committed pre-thymic T cell precursors which function as regulators in the hemopoietic system and as such mediate resistance to hemopoietic tumors. We will attempt to confirm that NK cells regulate EBV-induced B cell proliferation and examine by co-cultivation experiments the influence of various human T cell subpopulations on myelopoiesis and erythropoiesis. In addition we will attempt to determine the prognostic significance and therapeutic implications of all phenotypes defined by a panel of cell surface markers and to develop a mouse hybridoma which produces monoclonal antibody to human terminal transferase, an extremely useful leukemia cell marker.

BIBLIOGRAPHIC REFERENCES: Kaplan, J., and Callewaert, D.M. Expression of human T lymphocyte antigens by natural killer cells. J. Natl. Cancer Inst. 60:961-964, 1978. Callewaert, D.M., Lightbody, J.L., Kaplan, J., Jaroszewski, J., Peterson, W.D., Jr., and Rosenberg, J.C. Cytotoxicity of human peripheral lymphocytes in cell-mediated lympholysis: antibody-dependent cell-mediated lympholysis and natural cytotoxicity assays after mixed lymphocyte culture. J. Immunol. 121:81-85, 1978.

286. KILLER T-CELLS WITH EBV-RELATED SPECIFICITY IN INFECTIOUS MONONUCLEOSIS

Svedmyr, E., Karolinska Institutet, Dept. of Tumor Biology, *Solnavagen 1, S10401 Stockholm 60, Sweden*

We have found killer T-cells with EBV-related specificity in IM cases (EBV-caused). The antigen responsible for target sensitivity has been termed LYDMA. We intend to study primary EBV infections, with IM or with no or noncharacteristic symptoms, picked initially as susceptible IM contacts, and compare development of LYDMA-specific cytotoxicity, serology and clinical findings. Further characterization of LYDMA, killer cells as well as investigation of a possible role of 'syngeneic restriction' will be attempted.

287. NATURAL AND TISSUE-SPECIFIC IMMUNITY TO HUMAN NEOPLASMS

Saksela, E. J., Wahlstrom, T. R., Timonen, T., Paasivuo, R., University of Helsinki, Dept. of Pathology, *Fabianinkatu 33, SF00290 Helsinki 17, Finland*

Methods have been developed for enrichment and characterization for human natural killer (NK) cells. The morphology, functional characteristics and certain surface characteristics of human NK cells have been described.

The effect of interferon on the fractionated human NK cells has been studied. We have demonstrated that NK cells produce interferon when contacted with tumor target cells and the produced interferon recruits an initially non-cytoxic 'pre-NK' cell population to killer cells with the properties of NK cells. There is thus, within the NK system itself a self-amplifying positive feed-back mechanism which may be potentially important as a first line of defence against tumor cells.

Using the morphology of the human NK cells as markers we have improved the fractionation methods and obtain 85-90 percent pure fractions of such cells for continuation studies.

The presence of NK cells within human solid tumors has been investigated and the program on the specific immunological characteristics of normal and neoplastic glial cells continued. Various other tumor markers have been investigated and also applied to clinical diagnostic work.

BIBLIOGRAPHIC REFERENCES: Timonen, T., Ranki, A., Hayry, P. and Saksela, E.: Human natural cell-mediated cytotoxicity against fetal fibroblasts. III. Morphological and functional characterization of the effector cells. Cellular Immunol. 48: 121-132, 1979. Timonen, T., Saksela, E., Ranki, A.-M. and Hayry, P.: Fractionation morphological and functional characterizaton of effector cells responsible for human natural killer activity against cell line targets. Cellular Immunol. 48: 133-148, 1979.

288. LYMPHOCYTE SENSITIZATION TO TUMOR ANTIGENS IN VITRO

Golub, S. H., University of California, Los Angeles Campus, School of Medicine, Dept. of Surgery, *2025 Zonal Ave., Los Angeles, California*, 90024, United States of America

The summary for this project is under entry no. 57.

289. PROTEINASES OF HUMAN NATURAL KILLER CELLS

Hudig, D., Mendelsohn, J., University of California, San Diego Campus, School of Medicine, Dept. of Medicine, *P.O. Box 109, La Jolla, California*, 92093, United States of America

Synthetic, plant and plasma antiproteinases inhibit the natural cytotoxicity (NK) of human peripheral blood lymphocytes to tumor cells. The role of proteinases in NK will be studied by in vitro substrate competition of NK and by identification of proteinases selectively active during NK.

Class and substrate specificities of the proteinases will be determined by substrate competition of NK and by use of class-specific inhibitors. Identification and characterization of NK-specific proteinases will be made by radiolabeling of proteinases, and examination to see if they are unique, or in elevated concentration, during NK. Proteinases will be labeled with 3H DFP, or by 125 I after isolation of plasma antiproteinase-proteinase complexes formed during NK. Characterization will include determination of molecular weight and isoelectric focusing point, localization in the NK incubation medium or in subcellular components, and approximate quantitation by specific radioactivity and micro-Kjeldahl N analysis.

The plasma alpha 1 antiproteinases that are most potent in inhibition of NK will be isolated and identified. Discrimination between effects on NK induction and/or mediation will be made by experiments to delay induction and to block NK of pre-induced cells. Time lapse cinematography will help determine whether the plasma antiproteinases inhibit the early interaction between monocytes and lymphocytes which results in induction of lymphocyte NK activity and attachment to tumor cells, or whether the plasma antiproteinases affect only the later events of tumor cell cytolysis.

This study will contribute to better understanding of the mechanism of cell-mediated cytotoxicity, and of the effects of the cellular environment upon cells cytotoxic to tumors.

290. NATURAL KILLER CELLS - GENETIC CONTROL AND ROLE

Klein, E., Kiessling, R., Ahrlund Richter, L., Masucci, G., Masucci, M. G., Karolinska Institutet, Dept. of Tumor Biology, *Solnavagen 1, S10401 Stockholm 60, Sweden*

This project is presently focused on the following problems: 1. The relationship between natural killing (NK) and activated lymphocyte killing (AK) against K562 and T cell lines, as contrasted against B-cell derived lines. 2. Interferon activation of cytotoxicity (IAK) a) differential sensitivity of different established lines; b) preferential killing of allogenic, compared to autologous tumor biopsy cells. 3. The natural attachment (NA) phenomenon. 4. The increase of the NK-sensitivity of EBV carrying cells upon their entry into the viral cycle.

291. CHARACTERIZATION OF HUMAN NATURAL KILLER CELLS

Tarkkanen, J., Saksela, E., Timonen, T., University of Helsinki, Dept. of Pathology, *Fabianinkatu 33, SF00290 Helsinki 17, Finland*

OBJECTIVE: Human natural killer cells (NK-cells) and interferon are currently under worldwide laboratory investigation. This results from their possible role in the genesis and treatment of cancer. We will study the NK-cells of both healthy donors and cancer patients, and examine the problem of how cancer cells escape surveillance by the immune system in vivo.

APPROACH: We have developed techniques in our laboratory to isolate human NK-cell, taking advantage of the fact that NK-cells adhere to the surface of sensitive target cell (Timonen et al.). Utilizing this adsorption-elution technique we will further characterize the properties of NK cells. We have demonstrated that NK-cells display autologous restriction. This can, however, be abolished through contact with cancer cells (Tarkkanen et al.). We are currently studying the effect of interferon on these aspects. We will also initiate a project with cancer patients to determine whether the reactivity of their NK-cells differs from that in healthy donors.

292. SEPARATION OF RAT, DOG, AND HUMAN LYMPHOCYTE POPULATIONS

Sjogren, H. O., Lunds Universitet, Dept. of Tumor Immunology, Wallenberglaboratoriet, *Fack, S22007 Lund 7, Sweden*

An aqueous two-phase counter current distribution system for lymphocyte separation has been worked out and applied for separation of rat, dog and human lymphocyte populations. Separation of several functionally different lymphocyte subpopulations has been achieved. This technique is used to isolate lymphocytes with natural killer (NK) activity for their specific analysis and to free effector of tumor selective effects from the NK cells.

Unique and embryonic antigens associated with chemically induced rat intestinal carcinomas are studied in relation to tumor rejection, immunoprevention, therapy and diagnosis. Various immune assays are used to analyze tumor immunity in animals and in human melanoma patients.

293. ANTIGEN RECEPTOR OF CONTINUOUS T KILLER CELL LINE

Dennert, G., Salk Inst. for Biological Studies, Cancer Biology Laboratory, *10010 N. Torry Pines Blvd., P.O. Box 1809, San Diego, California*, 92112, United States of America

The goal of the proposed project is to characterize permanent lines of allospecific cell lines of thymus derived lymphocytes (T cells) with the ultimate aim to explore the specificity and nature of T cell receptors. Lines of allospecific T cells have been established by repetitive antigenic stimulation. The antigenic specificities recognized by two lines have been determined as well as the relation of Lyt phenotype and function. Recently, one of the lines has been cloned and therefore the properties of these clones are being examined. Idiotype specific antibodies able to bind to monoclonal antibody specific for the antigens recognized by the T cell lines are being prepared in order to assess whether the idiotypes of antibody and T cell receptors are shared. Experiments are also in progress to explore the in vivo effects of these cell lines as well as to characterize a factor released by these lines and able to trigger bone marrow derived cells for antibody synthesis.

BIBLIOGRAPHIC REFERENCES: Dennert, G. (1980). H-2 restricted T-cell-mediated cytotoxocity: Rediscovering allo-H-2 reactivity? Cell. Immunol., 49, 12. Dennert, G., R. Hyman, J. Lesley, and I. Trowbridge (1980). Effects of cytotoxic monoclonal antibody specific for T200 glycoprotein on functional lymphoid cell populations. Cell. Immunol., in press.

294. ONTOGENY AND CHARACTERIZATION OF NATURAL KILLER CELLS

Kim, Y. B., Sloan Kettering Inst. for Cancer Research, Dept. of Ontogeny of the Immune System, *145 Boston Post Rd., Rye, New York*, 10580, United States of America

It has been suggested that natural killer (NK) cells may be of prime importance in immune surveillance or natural resistance against neoplasms alternate to the clinical immune system. The unique immunologically 'virgin' germfree, colostrum-deprived piglets and naturally farrowed, colostrum-fed piglets from the specific pathogen-free (SPF) Minnesota miniature swine model will be used to investigate the following four specific aims: 1) define cellular nature of effector cells for natural killing; 2) relationship between NK cells and killer (K) cells for antibody-dependent cellular cytotoxicity (ADCC); 3) ontogenic development of NK cells; and 4) factors influencing development and/or activation of NK cells as well as regulation of NK activity. A short-term (2 1/2-4 hr) 51Cr-release assay method will be used for cytolytic effector activity for both NK and K cells with 51Cr-labeled human myeloid tumor cell line K562 or human T cell line HSB and TNP-conjugated human B cell line SB (TNP-SB) coated with IgG porcine anti-TNP, respectively. Because the immunologically 'virgin' piglets are completely devoid of 'background' NK activity, but have K cells for ADCC, we have an excellent opportunity to study the ontogenic development of NK cells and to definitively characterize the cellular nature of NK cells and K cells. In addition, the enhancing or suppressing factors for development, differentiation and/or activation of these important effector cells will be defined, which may eventually lead to manipulation of NK cells against neoplasia.

295. CHARACTERIZATION OF NATURAL KILLER CELLS

Tai, A., University of New Mexico, School of Medicine, Dept. of Pathology, *University Hill N.E., Albuquerque, New Mexico*, 87131, United States of America

Endogenous as well as augmented NK reactivities are being characterized with respect to the following: 1) genetic control of the phenomena which involve the study of endogenous and augmentable NK reactivities in parental, F1, F2 and backcross mice of differet genetic backgrounds, 2) determination of strain dependence versus target dependence by the use of different tumor target - mouse strain combinations, 3) separation (purification) of effecter cells by a number of biophysical criteria, e.g., size, adherence to nylon wool, G-10 sephadex, plastic, 4) serological characterization of NK population(s) by the use of specific anti-NK reagents, 5) sorting studies by the use of flow cytometry in a FACS-III system based on light scatter and serological markers.

296. NATURAL KILLER CELLS

Haller, O., Universitat Zurich, Inst. for Medical Microbiology, Experimental Microbiology, *P.O. Box 8028, Zurich, Switzerland*

OBJECTIVE: Characterization of mouse natural killer cells, their specificity and their role in vivo.

APPROACH: Spleen cells of young adult mice will be enriched for natural killer cells by treatment with carbonyl iron and magnetism followed by filtration through anti-Ig bead columns and subsequent incubation with anti-Thy serum plus complement. These cells will be analyzed with regard to: a) cell surface markers using allo- or heteroantisera of various specificities, b) binding of various lectins, c) rosette formation with a panel of malignant or normal cell types, d) killing capacity in comparison to other effector cell systems such as K cell or macrophage killing. Means to stimulate or abrogate natural killer cell activity in the intact animal will be investigated and used to further define the in vivo role of this cell type.

PROGRESS: New project.

297. IMMUNOGENETICS OF NK-1(PLUS) NATURAL KILLER CELLS

Koo, G. C., Sloan Kettering Inst. for Cancer Research, Laboratory of Cell Surface Immunogenetics, *410 E. 68th St., New York, New York*, 10021, United States of America

Spleen cells of untreated mice lyse BALB.RL male 1 (RL.1) leukemia cells in vitro. We have adopted this as a standard system for immunogenetic studies of natural killer (NK) cells. For our purposes we define the NK population as the cell set with RL.1 lytic capacity and surface phenotype Nk-1(plus). In preliminary work we have (1) established methods to enrich this NK cell set from heterogeneous populations; (2) adapted the Protein-A-SRBC rosette assay, which allows recovery and enrichment of both marker-positive and marker-negative viable cells, for use in the Nk-1 system; (3) established the Nk-1 phenotypes of 20 inbred strains; (4) determined that Nk-1 phenotypes are not related to levels of NK activity in different mouse strains, and (5) obtained evidence of a pro-Nk-1 cell set inducible for Nk-1 expression in vitro, permitting various uses of differentiative induction assays in the Nk-1 system. We propose (1) to define further the surface phenotype of this NK cell set in terms of expression of known immunogenetic surface markers; (2) to determine the differentiative origin of the NK cell set and its developmental relation to other cell sets; (3) to study the segregation of the Nk-1a (Nk-1.1) allele, map the Nk-1 locus, and to find alterative Nk-1 alleles and Nk-1 alloantigens; and (4) to determine whether C-type viral components are involved in NK activity in this model system. Thus we aim to determine the ontogeny of the Nk-1(plus) cell set and to elucidate the specificities of its RL.1 lytic activity.

298. NATURAL CYTOTOXIC CELLS IN THE MOUSE

Stutman, O., Memorial Sloan Kettering Cancer Center, Dept. of Cellular Immunobiology, *1275 York Ave., New York, New York*, 10021, United States of America

OBJECTIVE: To determine if the natural cytotoxic (NC) cells detected in nude normal mice in vitro against targets derived from solid tumors (chemically induced fibrosarcomas, virally induced mammary adenocarcinomas, etc.) may have in vivo significance. These studies will include: 1) characterization of the effector cells; 2) characterization of the susceptible or resistant tumor cells; 3) determination of the mechanism of the in vitro activity of the natural cytotoxic cells and 4) determination of the in vivo significance of the natural cytotoxic response in mice. The in vivo correlations will also include attempts to abrogate natural cell-mediated cytotoxicity (a thymus-independent putative candidate for the antitumor surveillance mechanism) and to determine the effects of such procedures on tumor development and biological behavior of spontaneous and/or transplanted tumors. To a large extent, this is a re-evaluation of the immune surveillance concept, but as a thymus-independent proposition.

299. STUDIES ON THE SPECIFICITY OF NATURAL KILLER CELLS

Henney, C. S., Fred Hutchinson Cancer Research Center, *1124 Columbia St., Seattle, Washington*, 98104, United States of America

In recent studies aimed at defining the selective lysis of tumor cells by murine natural killer (NK) cells, we have isolated from a methyl cholanthrene-induced DBA/2 lymphoma L5178, two clones of cells, one particularly susceptible to lysis by NK cells, the other almost totally resistant. Serological analysis showed no marked differences in cell surface alloantigen display on the two cell lines. Similarly, they were indistinguishable in their susceptibility to alloimmune cytotoxic T cells. The cells thus appear to be NK susceptibility variants. Preliminary biochemical characterization of the L5178 variants revealed that the susceptible cell line showed marked increases in the cell surface display of asialo GM2 glycolipid; the unsusceptible variant showed undetectable amounts of this compound. These differences were established both biochemically (using CHCl3: MeOH extraction followed by thin-layer chromatography and resorcinol: orcinol staining) and immunochemically using antisera specific for asialo GM2. In the work proposed, we wish to characterize more completely cell surface macromolecule display (protein, glycoprotein, glycolipid) on the cell variants of L5178 which differ in their susceptibility to NK cells. In an attempt to address the hypothesis that NK cell populations might be directed against cell surface glycolipids we intend: a) to increase glycolipid display on insusceptible cells by fusion with liposomes of defined content, b) to systematically 'block' glycolipids on susceptible target cells with defined antisera and to note effects on NK cell mediated lysis, and c) to assess the susceptibility of glycolipid containing liposomes to NK attack.

In a more general approach to the issue of the specificity of NK cell mediated attack we intend to use controlled protease and glycosidase digestion of the target cell surface and to assess the effects of such treatment on NK cell mediated lysis.

2. K AND NK CELLS IN TUMOR IMMUNITY

300. NATURAL AND ANTIBODY-DEPENDENT CYTOTOXIC ACTIVITY OF HUMAN LEUKEMIA CELLS

Hollan, S. R., Petranyi, G., Benczur, M., National Inst. of Hematology & Blood Transfusion, *Daroczi Ut. 24, P.O. Box 44, H1502 Budapest, Hungary*

OBJECTIVE: The natural (SLMC) and antibody-dependent (ADCC) cytotoxic activity is the property of lymphoid cells belonging to 'K' or 'O' populations respectively.

Our investigations cover: the determination of SLMC in acute leukemias; the detection of antibodies or factors in the serum that may block or increase the SLMC and ADCC activity; and the correlation between SLMC and ADCC and the clinical state of the patients.

APPROACH: The SLMC and ADCC activity is measured by chromium-release technique using K-562 cell line and chicken red blood cell targets. Separation of T,B, and 'O' cells is also employed to detect the cytotoxic activity of subsets.

PROGRESS: It is hoped that results of these studies would render data of diagnostic value, which may aid in monitoring the effects of therapy.

301. NATURAL AND ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY IN MAMMARY CARCINOMA PATIENTS

Bakacs, T., Garam, T., Svastits, E., Ringwald, G., National Inst. of Oncology, Tumor Immunology Section, *P.O. Box 21, H1525 Budapest, Hungary*

OBJECTIVE: Natural cell-mediated cytotoxicity (NCMC) and antibody dependent cytotoxicity (ADCC) may be envisiged as a second line of defense to specific T cell mediated disease related cytotoxicity. There are, in fact, several reports which claim NCMC and ADCC decline as malignant disease progresses. Whether this is a result of or the cause of the disease is a matter for discussion, but these reactions could be used to monitor general immune responsiveness in malignant patients.

NCMC and ADCC reactions measured in the classical way did not show the sensitivity expected. It was discovered that lymphocyte cytotoxic activity functions much like enzymes do. In other words cytotoxic activity of a constant number of lymphocytes can be increased until a plateau is reached by increasing the target cell number. The maximal activity measured this way we called cytotoxic capacity. Obviously this capacity is underappreciated if tested in the classical way. Therefore we have started to measure NCMC and ADCC activities of mammary carcinoma patients in the cytotoxic capacity test. We collect blood and test lymphocyte cytotoxic capacity before, during and after treatment, and seek a correlation between activities measured and treatment. Experiments have already started and more than 40 patients have been tested. We will terminate this project at the end of the year.

APPROACH: Peripheral blood of mammary tumor patients is collected in heparinized tubes. Mononuclear cells are separated according to Boyum. Macrophages are removed according to Jondal et al. Thereafter, lymphocytes are tested against K-562 myeloid leukemic cell line (NCMC) and against sensitized human Rh positive red blod cells (ADCC) in a 51Cr-release test.

302. THE HUMAN SPONTANEOUS KILLER (SK SYSTEM) - IN VITRO STUDIES ON NATURAL AND GENERATED KILLER CELLS IN PERIPHERAL BLOOD LYMPHOCYTES

Targan, S., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Research Service, *Wilshire & Sawtelle*

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Over the last year we have continued to look at activation of the natural killer (NK) cell system using a single cell assay. We have clearly defined a 'pre-NK' population of cells which bind to NK-target cells but need an external signal to become active cytotoxic cells. Besides interferon, we demonstrated that 3'5' GMP, acetylcholine, imidazole, and moderate exercise can recruit this same population of NK cells. Furthermore, we have shown that the same concentration of Prostaglandin E2 (PGE2) can both activate as well as inhibit the NK cytotoxic process. We also have data which suggest that a given number of NK cells can be activated to kill more by enhancing their ability for target cell lytic interactions. Finally, we have demonstrated the presence of NK cells in the intestinal mucosa and their ability to be augmented by interferon to kill autologous epithelial cells. Two manuscripts are in press and three have been submitted for publication from this material.

303. NATURAL KILLING IN HUMANS

Koren, H. S., Duke University, School of Medicine, Dept. of Microbiology & Immunology, *Box 3711, Durham, North Carolina*, 27710, United States of America

Natural killing (NK) is described as the cytotoxic activity of lymphoid cells occurring in the absence of any apparent stimulation against certain target cells. In the human, the activity seems to reside in a minor mononuclear lymphoid cell population having receptors for the Fc portion of Ig, lacking detectable SIg and phagocytic activity. The specificity of NK is unclear though the killing is selective. The object of the work proposed here is to investigate the exact nature and possibly heterogeneity of the effector cells involved in NK, and their specificity. Characterization of NK cells will be performed with NK cell enriched population obtained by the application of specific cellular and subcellular immunoadsorbents. We will search for cell markers characteristic of NK cells by using sera from sensitized individuals (multiparous women and hypertransfused patients). The possible relationship of NK to antibody dependent cellular cytotoxicity (ADCC) will be explored by using normal cells and cells from X-linked agammaglobulinemia patients that lack ADCC but have NK activity. In light of the possibility that NK has in vivo relevance to tumor resistance we will evaluate the level of NK in material obtained from ovarian cancer patients. NK activity of patients will be compared to controls by testing the effector cells against autochthonous and allogeneic tumor targets. Genetic factors associated with NK will be studied using families with known genetic background. The overall aim is to clarify the nature and specificity of the effector cells and their possible role in ovarian cancer.

304. ON THE RELEVANCE AND FUNCTIONS OF NATURAL KILLER CELLS

Wigzell, H. L., Nilsson, K., Lundin, L. G., Ohrn, A., Gidlund, M., Axberg, I., Perlmann, P., Harfast, B., Uppsala Universitet, Dept. of Immunology, *Box 256, S75105 Uppsala, Sweden*

The aim of the present proposal is to obtain more information on natural killer (NK) cells in mice and humans. We would like to develop reagents allowing the selective purification of NK cells or NK susceptible targets from heterogeneous populations and likewise to purify the relevant molecules responsible for the interactions between NK cells and their susceptible targets. We would also like to determine the optimal conditions for via externally administered reagents such as interferon maintain high NK levels in vivo. Finally, we would then analyze the actual in vivo relevance of NK cells as assessed in tumor resistance, marrow grafting or certain infectious diseases. The methods involve lectins of specific binding properties, cellular fractionation procedures taking advantage of surface markers on subgroups of cells, genetical and surgical maneuvers so as to create histocompatible animals with distinctly different NK levels for further studies.

305. KILLER LYMPHOCYTE DAMAGE TO TARGET CELL MEMBRANES

Henkart, P. A., Simone, C. B., Kerrigan, D., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Immunology Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

As reported previously, both complement and ADCC effector cells release markers resealed inside red cell ghosts with a very strong size dependence, such that markers below a defined cut-off size are released while all those which are larger are not. This sieving effect was also observed when the entry of protein markers into complement treated ghosts was measured, with the same protein molecular weight cut-off as was observed for marker efflux. The possibility of inhibiting the passage of molecules through the pore by blocking with anti-complement component antibodies was then tested. Anti-C3, -C5, -C8, and -C9 were tested. Anti-C5 blocked marker entry greater than 90% at dilutions to 1/1000. None of the other antisera blocked at 1/10. Anti-C5 did not block passage of markers through pores created by detergent. This approach is currently being extended to ADCC pores.

BIBLIOGRAPHIC REFERENCES: Simone, C.B. and Henkart, P.A.: Permeability changes induced in erythrocyte ghost targets by antibody-dependent cytotoxic effector cells: evidence for membrane pores. J. Immunol. 124: 954-963, 1980.

306. PSYCHOLOGICAL FACTORS AS RELATED TO INTERFERON AND NATURAL KILLER CELL ACTIVITY

Fox, B. H., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Field Studies & Statistics Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Earlier findings suggest that there is a relationship between self-reported psychiatric symptoms and natural killer cell (NK) activity. Further work demonstrated that the self-reports to a considerable degree reflected trait chracteristics in psychological tests. In an effort to determine whether interferon might have stimulated the appearance of the NK activity of those with high levels, an interferon analysis of the stored sera will be carried out.

307. ANTIGEN SPECIFIC NATURAL KILLER CELL REACTIVITY AND SUPPRESSION IN MICE NEONATALLY INFECTED WITH MMTV

Lane, M., Sidney Farber Cancer Center Inc., Division of Immunogenetics, *44 Binney St., Boston, Massachusetts*, 02115, United States of America

Mice neonatally infected with mouse mammary tumor virus (MMTV) are capable of antigen specific natural killer (NK) responses. As we have previously demonstrated, prior to 16 weeks the C3H/HeJ female possesses natural killer cells capable of recognizing MMTV gp52 ad MuLv gp71. After 16 weeks of age, a response to MMTV p28 is also present. These responses may be detected in vitro with the use of tumor target cells which express these antigens, and may be blocked in vitro by the addition of specific purified viral proteins and glycoproteins. Females of this strain bearing spontaneous mammary tumors of 15x15 mm or greater, demonstrate reactivity only against MuLv gp71. The spleens of these tumor-bearing females possess a cell capable of specifically suppressing NK responsiveness to antigens of MMTV, but not MuLv. We propose to investigate the significance, in this strain, of the arising of an MMTV p28 response and its relationship to antigen expression of preneoplastic nodules: to define the precise timing of decline of immune responsiveness to MMTV gp52 and p28 during tumor development; and to investigate the nature of the antigen-specific suppressor cell described in this system. Based on findings in these studies we will institute modes of therapy with purified antigens of MMTV. These treatments will be designed to alter pre-existing host levels of immune responsiveness to benefit the host in the prevention of tumors.

308. IN VIVO ROLE OF NATURAL KILLER CELLS

Kiessling, R., Karolinska Institutet, Dept. of Tumor Biology, *Solnavagen 1, S10401 Stockholm 60, Sweden*

These studies are aimed at understanding the role of NK cells as a first line of defense against tumor cells and against virus infections. Studies are being performed with the mouse mutant beige, shown to be selectively devoid of NK activity. These mice were shown to resist transplantable tumors less well than control mice, as measured by transplantation assays and by short-term elimination of radiolabeled i.v. injected tumor cells. They are now being analyzed for their resistance to spontaneous tumors and to primary tumor induction with DMBA and with

MLV virus. We have also analyzed the possibility that NK cells may be of importance in regulating or eliminating certain primitive cell types from the organism. In support of this notion, NK cells can kill a subclass of immature cortical thymocytes, which occur mainly in very young mice of NK low reactive strains. Interferon was shown to play a major role in modulating levels of thymocyte sensitivity to NK lysis. Recently, these studies were extended to a human system where human bone marrow, particularly when taken from fetuses, was found to be NK sensitive. We are now investigating if the colony forming granulocytic stem cells are among the cells killed by NK cells in human BM.

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309. REGULATION OF THE NATURAL KILLER (NK) CELL RESPONSE TO TUMORS

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A subpopulation of lymphocytes, called natural killer (NK) cells, may provide a first line of defense against newly arising malignancies. It is the purpose of the present investigation to gain a much better understanding of the events leading to tumor cell destruction and the factors regulating NK cell function. In part I, we hope to elucidate the nature of the signal which triggers the NK cell subsequent to target cell recognition and contact. The role of cyclic nucleotides (cAMP, cGMP), or various modulators thereof (prostaglandins, histamines, cholera toxin, theophylline, imidazole or cholinergic agonists), will be investigated in relation to their role in NK cytolysis. We will also evalute the effects of hormones (insulin, adrenalin, corticosteroids) since: (1) many hormones elevate cAMP levels in cells, (2) hormones could provide a model for NK regulation in vivo and (3) hormone imbalances are implicated in some cancers. In the second part of this study, we will investigate the role of complement in the NK lytic pathway.

In part III, we will investigate NK function in a new and exciting mutant strain of mice which appears to have a selective defect in NK killing, whereas cell-mediated lysis by T cells, K cells and macrophages is normal, and humoral immunity is unimpaired. The selectivity and intrinsic nature of the defect will be firmly established and we will indentify the aberrant gene product in the lytic pathway. Furthermore we will attempt to restore the defect and the mice will be used to conclusively establish the role of NK cells in resistance to tumor challenge, oncogenesis and spontaneous tumor development in vivo. We believe that this strain could be as important to tumor immunology as the nude mouse and should therefore be thoroughly investigated. In the final part of the project we will attempt to raise a monoclonal antibody against purified NK cells in order to define a much needed NK surface marker.

We believe this project is essential to provide conclusive proof that NK cells are involved in surveillance against neoplasia and to elucidate the steps in the NK lytic pathway. This knowledge will provide a basis for our eventual understanding and possible control of the host response to tumors.

310. GENETICS AND REGULATION OF NATURAL CELL-MEDIATED CYTOXICITY

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The objective of this research is to elucidate the genes, cells, and mechanisms involved in the regulation of natural cell-mediated cytotoxicity (NCMC). We will examine in the mouse both non-H-2 and H-2 genetic control of NCMC using congenic and recombinant inbred strains of mice. Gene complementation will be investigated in one system. An optimal protocol for in vivo and in vitro augmentation of NCMC will be established and applied in genetic analyses. The cellular requirements and characteristics of in vitro activation and suppression of NCMC will be investigated. Methods for selectively depleting or isolating natural killer (NK) cells will be developed so that NK cells can be added or removed from cultures in studies of the NK cell's role in T and B cell immune responses. Conventional antibodies and monoclonal-antibody-producing hybrid cell lines specific for NK-cell-associated antigens will be generated for this purpose. A nonhuman primate model for NCMC will be investigated. Family and in vivo augmentation studies will be performed. The characteristics of the primate NK cell and the specificity of NCMC against nonhuman lymphoblastoid cell lines will be investigated.

311. MODULATION OF NATURAL IMMUNE REACTIVITIES AGAINST TUMORS

Riccardi, C., Santoni, A., Barlozzari, T., Peruzzi, L., Bartocci, A., Cesarini, C., Carotti, C., Gosti, A., Universita degli Studi di Perugia, Inst. of Pharmacology & Pharmacognosy, *via del Giochetto, 06100 Perugia, Italy*

The aim of the present project is to study: 1) The possible involvement of NK cells in in vivo antitumor resistance. 2) The mechanisms involved in the regulation of NK activity. These approaches will be relevant to better understanding of the biological role of such reactivity and the possible relationships with other immune mechanisms.

In vivo role of NK activity: We have recently performed in vivo experiments measuring the rapid cytolytic (0-4 hrs) activity of non-immune mice against 125IUdR-labelled tumor cells. Such reactivity correlates with the in vitro NK activity, as measured in various organs (spleen, lungs, etc.) by different criteria: a) Strain distribution; b) age dependency; c) thymus independency; d) susceptibility to the treatment with various agents: antineoplastic drugs (adriamycin, cyclophosphamide, DTIC, ARA-C), interferon and interferon-inducers (Poly I:C, C. parvum, Pyran copolymer, MSV, tumor cells), antimacrophage agents (Silica and Carrageenans), 17beta-estradiol, Dexamethasone, lethal doses of irradiation. The correlations indicate that NK cells could play a role in in vivo antitumor immunity.

MODULATION OF NK ACTIVITY: Various agents (Pyran copolymer, Poly I:C, C. parvum, adriamycin, etc.) have been found to modulate NK activity. Stimulatory and inhibitory effects can be observed varying dose and time of administration. Some agents (adriamycin, Pyran copolymer, C. parvum) induce marked depression of NK activity at the time when high activation of macrophages is observed. At this time recovery of NK activity can be obtained by removal of nylon-adherent cells from the spleen of depressed mice. The suppressive activity can be transferred in vitro by a cell population showing the following characteristics: a) Plastic-adherence; b) radioresistance (2000-5000R); c) ability to phagocytize latex particles; d) adherence or phagocytosis of carbonyl iron; e) resistance to treatment with anti-Thy I antiserum plus C. These results indicate that activated macrophages could be inhibitory for the lytic activity of NK cells.

312. ROLE OF NATURAL KILLER (NK) CELLS IN SURVEILLANCE AGAINST TUMORS IN CHICKENS

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OBJECTIVE: The principal objective of this project is to study natural killer (NK) cell activity in the chicken and determine if this activity influences virus induced tumorigenesis.

APPROACH: A short-term Cr-release assay to quantitate NK cell activity will be developed. Efforts will be made to select a target cell that would be most sensitive to lysis by NK cells. Various lines of chickens will be screened for NK cell expression in an effort to recognize high and low responders. The identity of NK cells and the factors that influence the expression of these cells will be determined. Experiments will be conducted to correlate NK cell expression with susceptibility or resistance to tumor development.

313. SPECIFIC ANTITUMOR ACTIVITY BY 'ARMED' LYMPHOID CELLS

Pollack, S. B., Fred Hutchinson Cancer Research Center, *1124 Columbia St., Seattle, Washington*, 98104, United States of America

As we demonstrated previously, lymphoid cells from nonsensitized syngeneic mice and decomplemented antiserum from tumor-immune mice interact to produce specific killing of tumor cells in a microcytotoxicity assay in vitro. Cytotoxicity requires the presence of both antiserum and effector cells. We propose to continue our in vitro studies of this phenomenon and to investigate the in vivo role of antiserum-dependent cellular cytotoxicity (ADCC) in the anti-tumor response.

Specifically, we will: (1) Further characterize the effector cells which mediate ADCC to tumor targets and the effects of immunostimulants on the effector cells. Specific immunoadsorbent columns, adherence columns and specific antisera will be used to purify K cells. Their morphology and ability to mediate ADCC to different types of target cells in 51Cr release and microcytotoxicity assays will be studied. (2) Use both chemically (3-methylcholanthrene) and virally (Moloney sarcoma virus) induced tumors in experiments to determine what role ADCC plays in vivo. Specific antibody plus K cells or other effector cells will be used in attempts to influence the course of tumor growth and to prevent metastasis. (3) Assess the effects on lymphoid cell populations of the non-Ig early-appearing arming factor which we previously described and which induces specific cell mediated cytotoxicity to tumor cells.

314. LYMPHOCYTE PRODUCTION AND TRAFFIC IN THE BONE MARROW

Rosse, C., Clagett, J. A., University of Washington, School of Medicine, Dept. of Biological Structure, *A-300 Health Sciences Ctr., SC-64, Seattle, Washington,* 98195, United States of America

Previous studies during the preceding grant period demonstrated that murine bone marrow contains a population of 'null' lymphocytes which respond to PHA blastogenesis, proliferation and expression of surface T cell phenotype. In contrast to peripheral PHA responsive cells, these small lymphocytes do not recirculate, are rapidly renewed and are present in nude mouse bone marrow but not in LN and SPL. The known characteristics of these 'pre-T cells' do not match any type of T cell precursor described by other assays and these cells may represent an alternative pathway of T cell development. The capacity of these cells for maturing under thymic influence or, independent of it, into cells capable of reacting in MLR, GVH and DTH to H2 antigens will be studied after they have been exposed to thymic epithelial monolayers, cAMP or to alloantigens in intact or thymectomized F1 hosts. It is known that bone marrow cells synergize with immune T cells in controlling the growth rate of tumors, and the role of bone marrow 'pre-T cells' phenomenon will be investigated using 3-methylcholanthrene induced sarcomas in mice. Following topical labeling of the bone marrow with 3H-TdR, the in vivo development of surface Thy-1 or brain associated antigen will be looked for on 3H-labeled cells that had migrated to the SPL, LN and THY, duplicating our studies on bone marrow derived B cells. The relationship of pre-T cells to prothymocytes and post-thymic T cell precursors will be investigated making use of experimental models such as thymic atrophy consequent upon 89Sr treatment and serial transfer of chromosomally marked, selected bone marrow populations. The relationship of pre B cells and pre-T cells will be explored in mu-suppressed and nude mice, as well as in regenerating bone marrow devoid of prothymocytes. Competition for expression of T or B cell surface phenotypes by bone marrow pre-B cells will be studied using specific and nonspecific inducing agents in vitro. The in vivo and in vitro studies will comprehensively characterize 'pre-T cells' produced in the BM at a sustained and rapid rate, and will define their status with respect to T cell production.

315. CANCER CHEMOTHERAPY AND MURINE NATURAL KILLER CELLS

Cohen, S., Nolan, J. P., Vladutiu, A., State University of New York, Health Science Center at Buffalo, School of Medicine, Dept. of Medicine, *3435 Main St., Buffalo, New York,* 14214, United States of America

This research proposal is focused on the study of the selective effects of anticancer drugs on the host's natural defense to tumors. The emphasis will be on identifying possible ways to pharmacologically modulate the murine natural killer system. The natural killer cell systems to be studied include spontaneous natural killer cells, cultured natural killer 'like' cells, and in vivo augmented natural killer 'like' cells (poly I/C, interferon). Comparisons will be drawn between drug effects on spontaneous natural killer cytotoxicity, and antibody dependent cellular cytotoxicity (against tumors) and between drug effects on natural killer cytotoxicity and in vivo protection against tumor growth. In addition, the effects of drugs on natural killer suppressor cells which modify the level of natural killer cytotoxicity will be studied. Drugs to be investigated include the alkylating agent cyclophosphamide and its metabolites phosphoramide mustard and 4-hydroxy cyclophosphamide and the anthracycline Adriamycin.

316. IMMUNOBIOLOGY OF CYTOTOXIC LYMPHOID CELLS

Henney, C. S., Nicolotti, R., Fred Hutchinson Cancer Research Center, Program in Basic Immunology, *1124 Columbia St., Seattle, Washington,* 98104, United States of America

In recent studies aimed at defining the selective lysis of tumor cells by murine natural killer (NK) cells, we have isolated, from a methyl cholanthrene-induced DBA/2 lymphoma L5178, two clones of cells, one particularly susceptible to lysis by NK cells, the other almost totally resistant. Serological analysis showed no marked differences in cell surface alloantigen display on the two cell lines. Similarly, they were indistinguishable in their susceptibility to alloimmune cytotoxic T cells. The cells thus appear to be NK susceptibility variants. Preliminary biochemical characterization of the L5178 variants revealed that the susceptible cell line showed marked increases in the cell surface display of asialo GM2 glycolipid; the unsusceptible variant showed undetectable amounts of this compound. These differences were established both biochemically (using CHCl3:MeOH extraction followed by thin-layer chromatography and resorcinol:orcinol staining) and immunochemically using antisera specific for asialo GM2. In the work proposed, we wish to characterize more completely cell surface macromolecule display (protein, glycoprotein, glycolipid) on the cell variants of L5178 which differ in their susceptibility to NK cells. In an attempt to address the hypothesis that NK cell populations might be directed against cell surface glycolipids we intend: a) to increase glycolipid display on insusceptible cells by fusion with liposomes of defined content, b) to systematically 'block' glycolipids on susceptible target cells with defined antisera and to note effects on NK cell mediated lysis, and c) to assess the susceptibility of glycolipid containing liposomes to NK attack.

In a more general approach to the issue of the specificity of NK cell mediated attack we intend to use controlled protease and glycosidase digestion of the target cell surface and to assess the effects of such treatment on NK cell mediated lysis.

317. KILLER CELLS IN IMMUNODEFICIENCY

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This is part of a broader project. A summary of this subproject is not available.

II. ROLE OF MONONUCLEAR PHAGOCYTES IN THE IMMUNE RESPONSE

A. ACTIVATION OF MONOCYTES AND MACROPHAGES

318. HUMAN MONOCYTE FUNCTION AND ACTIVATION

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The objectives are to elucidate the role of the monocyte in cellular immunity and to identify defects in this function in various diseases. The approach will be to study improved methods for generating lymphocyte mediators ('lymphokines') that affect macrophages and to improve present assays for monocyte function. In addition, new assays for monocyte function and activation are to be developed. Current plans include: 1) refinement and standardization of our newly described plasminogen activator assay for routine use in patients; 2) assessment of mechanisms of monocyte activation by MIF with particular emphasis on Ca(plus plus) influx and cyclic GMP metabolism;

3) study of Ca(plus plus) influx as an in vitro assay for MIF action on monocytes; 4) development of monocyte tumoricidal and lymphocyte activating factor assays.

Current studies have already demonstrated the involvement of Ca(plus plus) influx in MIF action and the feasibility of the plasminogen activator assay.

319. CHARACTERIZATION OF MONOCYTES, MACROPHAGES AND ACTIVATED MACROPHAGES

Klockars, M., Currie, G. A., Alexander, P., Chester Beatty Research Inst., *Clifton Ave., Belmont, Sutton, Surrey SM2 5PX, England, United Kingdom*

The role of macrophages in tumor immunity has become evident. A specific cytotoxic effect of activated macrophages on cancer cells has been described. The differentiation of monocytes and the activation of macrophages can be studied by means of their phagocytic activity, intracellular enzymes (arginase, lysozyme) and membrane characteristics. There is however, a substantial need for the identification of specific markers for these cells.

Radioactively labelled protein - carbohydrate complexes of the plasma-membrane has been used to study and identify normal and malignant leukocytes.

By the use of galactose oxidase - NaB 3Hu and periodate - NaB 3Hu labeling techniques the characterization of monocytes, macrophages and achivated macrophages is planned.

320. MECHANISMS OF MACROPHAGE ANTITUMOR ACTIVATION

Edelson, P. J., Children's Hospital Medical Center, *300 Longwood Ave., Boston, Massachusetts*, 02115, United States of America

Mouse peritoneal macrophages from inflammatory foci show a catalogue of characteristics which distinguish them from unstimulated cells, including (a) elevated pinocytic rate, (b) increased rate of synthesis and secretion of neutral proteases (plasminogen activator, elastase, collagenase), (c) ingestion of IgM-C3-coated particles, (d) low or absent levels of 5' nucleotidase activity. These characteristics will be used as criteria for judging the activation of macrophages in vivo and in vitro by lymphocytes responding to antigen, allogeneic cells, or tumor cell surfaces, in order to establish the mechanism of macrophage activation by cell-mediated immune reactions.

BIBLIOGRAPHIC REFERENCES: Edelson, PJ., and Cohn, A.Z. Endocytosis: Regulation of membrane interactions. Cell Surface Reviews 5:387-405, 1978.

321. MECHANISMS OF MACROPHAGE ACTIVATION AND TOXICITY

Ralph, P., Sloan Kettering Inst. for Cancer Research, Laboratory of Hemopoietic Development, *410 E. 68th St., New York, New York*, 10021, United States of America

This proposal will investigate mechanisms of antibody-dependent cellular cytotoxicity (ADCC) and activated macrophage cytolysis. Both red blood cells (RBC) and tumor targets will be studied with toxicity quantitated by release of 51Cr (chromium) or radiolabel from DNA. Various mouse peritoneal preparations and cloned macrophage cell lines known to differ in ability to mediate ADCC to RBC, ADCC to tumor targets and nonspecific activation to tumor lysis will be utilized to characterize physiological differences among subgroups of mononuclear phagocytes. The requirement for T or B lymphocyte participation in activation by microbial products will be tested in vitro. Existing models of activation by soluble factors derived from stimulated spleen cells will be used to study the role of the cell's physiology in this process. Metabolic and esterase inhibitors which we have shown to augment ADCC and other agents specifically blocking lysis but not target phagocytosis will be used to analyze the lytic mechanism. The effect of physical parameters such as temperature and type of assay vessel known to drastically alter the ratio of lysis to phagocytosis will be investigated to increase understanding of how macrophage effector functions can be modulated. Cell line macrophages will be synchronized to study cell cycle dependency for activation and effector functions. Antibody preparations of individual immunoglobulin heavy chain classes including monoclonal hybridoma antibody will be prepared to determine the function of class in directing ADCC or blocking macrophage toxicity. The overall emphasis of this proposal is on the physiology, heterogeneity and regulation of macrophage effector activity, rather than on characterization of lymphocyte stimulating factors or classes of susceptible and resistant targets.

322. ACTIVATED MACROPHAGE INDUCTION OF TUMOR CELL CYTOLYSIS

Connolly, K. M., Virginia Commonwealth University, Medical College of Virginia, Dept. of Surgery/Microbiology, *1200 E. Broad St., Richmond, Virginia*, 23298, United States of America

In our hands, activated macrophages (AM0) from C. parvum or pyran-treated mice are efficient effectors of tumor cell cytolysis. Flow microfluorometric analysis of tumor cell DNA following contact with AM0 demonstrated that tumor cell division occurred despite early shutdown of DNA synthesis and resulted in a 50% reduction of tumor cell DNA content. Since reductive tumor cell division in the absence of DNA synthesis may be related to the mechanism of AM0-mediated tumor cell cytotoxicity, we, wishing to examine this phenomenon on an intracellular and biochemical level, propose to: 1) Determine why AM0 inhibit contact-dependent cytolysis for neoplastic cells, while inducing only non-lethal suppression of DNA synthesis in normal proliferating cells. 2) Determine the intracellular mechanism(s) involved in AM0-induced, tumor cell selective, cytolytic activity and determine if an in vivo correlate can be observed. By incubating normal and neoplastic cells with cholesterol or lecithin lysosomes, we wish to examine the relationship between AM0-induced cytotoxicity and target cell membrane cholesterol content. Additional plans include: 1) Establishing whether AM0 membrane vesicles can be substituted for intact AM0. 2) Using synchronized normal and tumor targets to determine where in the cell cycle neoplastic cells are most vulnerable to AM0. 3) Identifying tumor cells with reduced DNA content at the in vivo site of AM0-mediated tumor regression.

323. ROLE OF MACROPHAGES IN RESISTANCE TO CANCER

Hibbs, J. B., Granger, D. L., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Infectious Disease Section, *500 Foothill Dr., Salt Lake City, Utah*, 84113, United States of America

We have noted cytotoxic activated macrophages (CM) inhibited the growth of neoplastic L1210 cells in vitro but L1210 cell death was minimal to nonexistent. L1210 cells injured by CM were separated from macrophages and studied in an isolated system. CM-injured L1210 cells had an absolute requirement for glucose or another glycolyzable hexose (mannose or fructose) for at least 40 hrs after removal from macrophages. If the culture medium lacked sufficient concentration of one of these sugars, CM-injured L1210 cells died within 4 hrs. Uninjured L1210 cells cultured alone or with peptone-stimulated macrophages had no such requirement and maintained complete viability in hexoseless medium. The hexose requirement of CM-injured L1210 cells could not be fulfilled by other naturally occurring monosaccharides, glucose or mannose derivatives, or substrates that can be oxidized by mitochondria. The concentration requirements for glucose, mannose, and fructose by CM-injured L1210 cells correlated with the concentrations required to support maximal glycolysis of these sugars by other murine ascites cells.

Interaction with CM led to inhibition of L1210 cell mitochondrial oxidative phosphorylation. This was supported by the findings that: (a) CM-injured L1210 cells had no Pasteur effect; their rate of aerobic glycolysis was the same as the rate of anaerobic glycolysis of uninjured L1210 cells. (b) Endogenous respiration of CM-injured L1210 cells was 15% of normal. Maximal inhibition of uninjured L1210 cell respiration by a specific mitochondrial poison (oligomycin) was nearly the same (13% of normal). It followed that CM-injured L1210 cells required hexose for chemical energy production via the glycolytic pathway. CM-induced mitochondrial injury occurred in five other neoplastic cell lines tested.

324. DEVELOPMENT AND FUNCTION OF ACTIVATED MACROPHAGES

Moore, M. A., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York*, 10021, United States of America

The functional heterogeneity of the macrophage population will be studied with respect to inducible or constitutive production of bioregulatory macromolecules which influence myelopoiesis and lymphopoiesis. 'Helper' macrophage populations will be assayed for capacity to produce stimulatory factors involved in the proliferation of granulocyte-macrophage, erythroid and megakaryocytic progenitor cells and mitogen-dependent T and B cell responses. 'Suppressor' macrophages will be assayed for production of prostaglandins, interferon, tumor necrosis factor and other activities of relevance to the anti-proliferative and anti-neoplastic role of macrophages. Biophysical separation and cloned macrophage cell lines will be used to facilitate characterization of functionally distinct macrophage subpopulations. Macrophage activating agents, including endotoxin and C. parvum, will be used to induce secretion of regulatory macromolecules in non-producer macrophage cell lines or macrophage subpopulations and biochemical characterization of these activities will be performed. Separate analysis of granulocyte macrophage regulatory interaction will be carried out with emphasis on the regulatory role of granulocyte derived lactoferrin and macrophage derived GM-CSF, endogenous pyrogen and prostaglandin E. Network analysis of regulatory interactions between macrophages and B and T lymphocytes, hemopoietic stem cells and progenitor cells will be carried out in order to define the central regulatory importance of macrophages in hemopoietic regulation and immune function. Finally, the effector function of macrophages in host defense against neoplasia will be studied from the standpoint of differential sensitivity of tumor target cells to suppressive factors produced by stimulated or activated macrophages.

325. STUDIES ON MACROPHAGE ACTIVATION

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OBJECTIVES: 1. Investigate the identity and function of cell populations that control macrophage activation in the development of anti-listerial resistance: a) identify and analyze the roles of the T-cell subpopulations involved in the generation of specifically mediated macrophage activation and delayed hypersensitivity in primary infection, using Ly antisera and the T cell deprived host (D-ATxBM); b) determine the identity and the source of the splenic cell population responsible for regulation of macrophage activation, and analyze its mode of action, using the splenectomized host. 2. Determine the cellular level of phenotypic expression of genetically controlled high or low levels of anti-listerial immunity: 1) study the effects of an adoptive transfer of purified cell populations between H-2 compatible congenic mouse strains exhibiting different levels of resistance; b) study the production and effect of lymphokines and macrokines in Listeria resistant and sensitive strains.

326. MACROPHAGE PROLIFERATION AND ACTIVATION

Hadden, J. W., Hadden, E. M., Coffey, R. G., Sloan Kettering Inst. for Cancer Research, Laboratory of Immunopharmacology, *410 E. 68th St., New York, New York*, 10021, United States of America

Delayed hypersensitivity in vivo is associated with macrophage proliferation which occurs concomitant to the development of lymphocyte sensitization. We have observed that antigen-stimulated guinea pig lymph node lymphocytes produce a soluble factor which induces the proliferation of nonimmune peritoneal and alveolar macrophages incubated as monolayers; partial purification of this factor using Sephadex chromatography indicates an approximate molecular weight similar to that of migration inhibitory factor (MIF); however, vacuum dialysis prior to chromatography removes MIF and, therefore, distinguishes between the two factors. Experiments are proposed to further purify this mitogenic factor and to analyze its effects on macrophage monolayers. The factor will be further compared to other factors known to act on the macrophage, particularly MIF, macrophage chemotactic factor, and colony stimulating factor. The action of this factor to induce macrophage activation in terms of enhanced microbicidal and tumoricidal activity is being examined. Peritoneal exudate macrophage subpopulations fractionated on discontinuous albumin gradients differ in the proliferative and activation responses to stimulation with MMF. Prostaglandin synthesis by macrophages appears to play a role in the regulation of MMF-induced proliferation. Based upon extensive experience with the lymphocyte and preliminary experiments with the macrophage, we plan to examine the mechanisms of action of this factor in relation to cyclic GMP generation and calcium influx, two key components of mitogen action. The responses of macrophages to stimulation by immunopotentiators, particularly those thought to act on cyclic nucleotide pathways, will be examined. The proposed experiments will contribute to the understanding of macrophage regulation and ultimately to immunotherapeutic endeavors to modulate the functions of this cell in enhancing resistance in the immunodeficient and/or cancer bearing patient.

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327. ENVIRONMENTAL DUSTS AND MACROPHAGE ACTIVATION

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It has recently become apparent that the environmental component of cancer is more significant that generally imagined. We propose to test noxious dusts and fibers, which are commonly found in the environment, for their ability to induce putative inflammatory mediators from mouse peritoneal macrophages. These molecules are the enzymes, plasminogen activator and collagenase, and prostaglandins. Several environmentally important materials will be used, with particular emphasis on asbestos. These fibers will be manipulated and physical measurements made to determine what structural and chemical characteristics are important for activity in our in vivo and in vitro test systems. We intend to study cellular control mechanisms for the synthesis of the mediators and hopefully to find drugs which could alleviate the deleterious effects of such fibers and dusts. We hope that our data may aid in the establishment of realistic control methods and standards for asbestos levels and types, as we will be placing emphasis on short asbestos fibers. It is also possible that our assays may be useful for detecting other harmful fibers and dusts in the environment. Because peritoneal mesotheliomas are a common form of mesothelioma in asbestos workers, we believe it relevant to be studying the interaction of asbestos with peritoneal cells.

328. BIOCHEMICAL BASIS OF MACROPHAGE ACTIVATION

Soberman, R. J., Harvard University, Boston Campus, School of Medicine, Dept. of Biological Chemistry, *25 Shattuck St., Boston, Massachusetts*, 02115, United States of America

The goal of this investigation is to comprehend the biochemical basis of macrophage activation. We will examine why mouse peritoneal macrophages activated by different mechanisms exhibit different biochemical and biological properties.

Macrophages will be activated by three different mechanisms: 1) intraperitoneal injection of sodium caseinate; 2) lymphocyte mediators; 3) direct contact with lymphocytes and antigen. Phagocytosis of 14C labeled starch particles and of 14C labeled dead tubercle bacilli will be measured. Glucose-1-14C and glucose-6-14C conversion to 14CO2, ecto-AMPase, and 5'-nucleotidase will be measured as parameters of activation. Tumoricidal activity will also be studied. We will then examine whether actinomycin D or cycloheximide treatment of macrophages can alter any of the parameters of activation listed above. We will study whether hydrocortisone or certain chemotherapeutic agents can affect activation.

In the series of experiments in which macrophages are incubated with lymphocytes and antigen the nature of the surface interaction will be explored. After exposure to boosting antigen, the lymphocyte surface will be exposed to trypsin, neuraminidase, and fucosidase. They will then be added to mouse peritoneal macrophages in culture. Macrophages will be modified by exposure to trypsin, neuraminidase, fucosidase, diazotized sulfanilic acid, and di-isofluorophosphonate. They will then be added to lymphocytes and boosting antigen in culture and the parameters of activation measured.

The role of cyclic nucleotides and divalent cations in the activation process will be studied.

329. INTERACTION OF MACROPHAGE ACTIVATING FACTOR WITH MACROPHAGES

Thomasson, D. L., Washington University, School of Medicine, Dept. of Radiology, *660 S. Euclid Ave., St. Louis, Missouri,* 63110, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

B. ANTITUMOR AND ANTIMICROBIAL ACTIVITIES OF MONOCYTES AND MACROPHAGES

330. MONOCYTES IN HODGKIN'S DISEASE AND MALIGNANT HISTIOCYTIC LYMPHOMA

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OBJECTIVE: To study the importance of monocytes in Hodgkin's disease and malignant histiocytic lymphoma with respect to pathogenesis and immunity.

APPROACH: 1. Monocytes will be isolated from peripheral blood and cocultured with cell lines derived from Hodgkin's disease and malignant lymphoma tissue, and with lymphocytic subpopulations. The interaction between the different cells will be studied. 2. Quantitative and qualitative examinations of monocytes will be carried out in patients with Hodgkin's disease and malignant histiocytic lymphoma.

The results will be compared with those obtained by examining patients with viral infections and autoimmune diseases.

331. ROLES OF PHOSPHOLIPID METABOLISM AND MONOCYTE FUNCTION IN TUMOR IMMUNITY

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Phospholipid metabolism was found to play a significant role in the in vitro recognition and destruction of tumor cell lines by human peripheral blood cells.

The ability of human peripheral blood monocytes to control proliferation by transformed cells was studied in normal individuals and patients with malignancy. Effector mechanisms of this growth inhibitory activity and its regulation were distinct from those usually attributable to other immune cells or effector molecules.

An enzyme-lined immunosorbent assay (ELISA) method to simply and rapidly detect the production of monoclonal antibodies directed against cell surface antigens was developed and implemented. This technique allowed selection of 'hybridomas' producing antibodies specific for human monocyte components.

332. MONOCYTES AND MACROPHAGES IN RESISTANCE TO CANCER

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A large body of in vitro evidence has accumulated which suggests that activated macrophages which come from hosts with chronic infections or which have been treated with immunomodulating substances might play an important role in prevention of tumor development or in resistance to existing tumors. Experiments will be performed to more clearly define the mechanisms underlying the adverse effects that activated macrophages exert on tumor target cells. The relative contributions that macrophage-mediated cytostatic and cytocidal effects make to this overall adverse effect will be studied, as will the ability of lymphocytes or their products to bolster (or suppress) the cytotoxic effects of activated macrophages. We will determine the cytotoxic efficacy of macrophages from different anatomical compartments, and we will define the most efficient methods of preferentially activating macrophages from these compartments. In humans, we will study in vitro peripheral blood monocytes and monocyte-derived macrophages, as well as tissue macrophages from the spleen, to determine their effects on established lines of normal and neoplastic human cell lines. Experiments will be performed to activate these human monocytes and macrophages in vitro to kill tumor cells by cultivating them in the presence of lymphocytes (or lymphocyte products) and mitogens or specific antigen.

333. ROLES OF ARMED MONOCYTES IN CONTROL OF METASTASIS

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This project is designed to investigate the roles of monocytes in limiting blood-borne metastasis of tumor cells, and to provide a valid basis for effective immunochemotherapy (or prophylaxis) of metastatic spreading.

Utilizing both metastasizing and nonmetastasizing syngeneic tumor lines of DBA/2J mice, host immunity is semi-quantitatively assessed in normal and tumor-bearing animals by injection of radio-labeled tumor cells and by measuring the rate of tumor cell destruction in various target organs of these animals, and the results are evaluated in relation to eventual tumor outgrowth in each organ.

Responses of tumors to chemotherapy and the metastasizing ability of tumor cells are also comparatively investigated.

Suppression by commonly employed chemo- and/or radiotherapeutic regimens of host defense functions and their possible restoration or augmentation by monocytic effectors are studied. Possible roles of T lymphocytes in such monocyte functions, as well as roles of monokines in activation of T cell activities, are investigated.

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334. MONONUCLEAR PHAGOCYTES IN HUMAN OVARIAN CARCINOMA

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The investigations proposed herein are designed to elucidate basic aspects of the immunobiology of monocytes and macrophages in human ovarian tumors. Mononuclear phagocytes will be isolated from the peripheral blood and ascitic tumors of patients with epithelial ovarian neoplasms and their cytolytic activity will be measured using in vitro tumor cell lines. Cytotoxicity of mononuclear phagocytes will be compared to that of peripheral blood monocytes and peritoneal macrophages of age-matched patients with non malignant diseases. Attempts will also be made to isolate and test macrophages from primary solid ovarian tumors. In parallel experiments, primary ovarian carcinoma cultures from peritoneal effusions and solid tumors will be analyzed for their susceptibility to macrophage cytotoxicity (lysis and/or stasis). We will also investigate whether stimuli, such as endotoxin and lymphokines, effective in experimental murine systems can enhance the cytocidal capacity of mononuclear phagocytes of ovarian carcinoma patients or control subjects. It is expected that the present study will provide experimental evidence relevant to a better understanding of the role of mononuclear phagocytes in the control of human neoplasia.

335. OPSONIC AND PHAGOCYTIC ACTIVITY DURING TUMOR GROWTH

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The role of opsonic alpha 2SB glycoprotein as a factor regulating macrophage activity during experimental tumor growth was investigated. Parallel studies have also been done in patients with lung cancer to assess the serum level of opsonic alpha 2SB glycoprotein following BCG immunotherapy. The opsonic protein from mouse, rat and human serum has been isolated and an immunoassay for its measurement has been developed. Opsonic alpha 2SB glycoprotein is identical to cold-insoluble globulin or plasma fibronectin. Both proteins have identical molecular weights, manifest ability to stimulate particulate phagocytosis, and demonstrate heparin dependency in terms of biological activity. In vivo assessment of opsonin levels during tumor growth utilizing the Sarcoma 180 tumor model in mice demonstrated that with an increase in tumor growth there is an elevation in immunoreactive opsonic protein, but a decrease in the bioassayable activity of the plasma. Reticuloendothelial phagocytic activity was also measured during Sarcoma 180 tumor growth and activation of macrophage clearance was associated with increased hepatic Kupffer cell activity. Patients with lung cancer after lung resection but prior to BCG therapy demonstrated heightened levels of opsonic protein as measured by immunoassay. This heightened level post-operation was typical of rebound hyperopsonemia after surgery. With BCG, a normalization of opsonin levels was observed. Thus, macrophage activity may be, in part, modulated by circulating opsonin or plasma fibronectin but the association of cell bound fibronectin with phagocytic activity is yet to be determined. Also, during tumor growth there may be an inhibition of bioassayable opsonin activity even in the face of elevation of immunoreactive protein.

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336. HUMAN MONONUCLEAR PHAGOCYTE-MEDIATED CYTOSTASIS AND CYTOTOXICITY TO LYMPHOCYTES AND TUMOR CELLS

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OBJECTIVE: To analyze the influence of human mononuclear phagocytes on target cell survival and growth.

APPROACH: Human monocytes and macrophages are cultured in vitro for up to 12 days. Mononuclear phagocyte-mediated influence on target cells is analyzed with radioactive isotope-labelled precursor incorporation or release from target cells. The influence of various agents (lymphokines, bacterial products) on mononuclear phagocyte-target cell interaction is investigated. Cytofluorographic investigations of target cells influenced by mononuclear phagocytes will be performed.

PROGRESS: Human monocytes develop cytostatic influence on lymphocytes and tumor cells during in vitro differentiation to macrophages. Cytostatic and cytotoxic activity is enhanced by lymphokines and bacterial lipopolysaccharides.

337. MACROPHAGE TUMOR CELL KILLING AND RED CELL CATABOLISM

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The overall purpose of my research is to understand the role macrophages play in control and elimination of cancer in experimental animals and humans. My studies and those of others have investigated mechanisms by which in vitro macrophage tumoricidal activity can be enhanced (treatment with endotoxin, lymphocyte-derived activating factors, or mild oxidants) or depressed (treatment with lipoproteins, corticosteroids, or red blood cells (rbc), hemoglobin (Hb), or iron). The potent inhibition caused by endocytosis of rbc, Hb, or iron is potentially important because it may help us understand mechanisms of macrophage activation and/or tumor cell killing, and because there are certain clinical states in which increased macrophage rbc catabolism and iron storage could be accompanied by depressed macrophage tumoricidal activity. The objectives of this proposal are: 1) to further characterize the molecular mechanisms by which rbc, Hb, and iron inhibit macrophage tumor cell killing in vitro, 2) to determine if in vivo alterations in rbc catabolism or iron status alter resistance to tumor growth or ability of macrophages to kill tumor cells in vitro, and 3) to determine if rbc, Hb, or iron alters human monocyte-macrophage tumor cytotoxicity in ways comparable to those noted for mouse macrophages.

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338. RESPONSE OF PHAGOCYTIC LEUKOCYTES TO TUMOR PROMOTORS

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12-O-Tetradecanoyl phorbol-13-acetate (TPA), the most powerful promoter in the phorbol series, initiates a series of metabolic events in granulocytes which are also activited during phagocytosis. Human promyeloid cells derived from a patient with acute promyelocytic leukemia do not respond, or respond poorly to TPA. During in vitro maturation, the cells achieve ability to respond to TPA as measured by an increase in hexosemonophosphate stunt activity and protease production. On this basis we propose to study the development of TPA-responsive systems during the maturation of human myeloid cells in vitro to determine: 1) Whether the enzymatic activities involved in the response of granulocytes to TPA can be detected prior to development of TPA sensitivity; 2) whether other tumor promoters can induce the same responses: 3) whether all of the enzymatic activities involved in the TPA response appear simultaneously or sequentially: and 4) whether anti-inflammatory glucocorticoids have any selective effect on the expression of the enzymes involved in the TPA response.

TPA-treated granulocytes are capable of inhibiting mitogen-induced lymphocyte activiation. We intend to determine whether this inhibitory activity is unique to TPA-treated granulocytes or whether it can be evoked by any agent which activates granulocytes to phagocytize, and to determine whether inhibition is mediated by cell-to-cell contact or by release of an inhibitory compound from treated granulocytes.

Finally, we will evaluate each of the TPA-induced reactions in granulocytes in terms of suitability for use as bioassays utilizing normal human cells for detecting agents with promoter potential.

339. MACROPHAGE RECOGNITION AND TUMOR CELL INTERACTIONS

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The human macrophage, an important component of the immune system, in large part functions to detect and damage foreign cells and to regulate certain immune interactions. The interaction between human macrophages and human erythrocyte target cells will serve as a study model for human macrophage-lymphoblastoid and myeloma cell interaction. Human erythrocytes coated with IgG or C3 will be bound to human macrophages, whereupon they become damaged (sphered) over time. The mechanism of the binding and sphering will be assessed by metabolic and pharmacologic (low concentrations of various lipid-soluble steroid analogues, levamisole) intervention, as well as by altering the macrophage membrane and the target erythrocyte (structurally, biochemically and immunologically). Comparisons will be made between isolated macrophage/monocyte subpopulations. Macrophage-lymphoblastoid cell and myeloma cell interactions will be examined as a model for macrophage-tumor cell interactions. The spontaneous interaction which we observe between these two former cells will be further defined. The effect of corticosteroids, levamisole, membrane cholesterol/phospholipid, immunologic activation and the requirement for specific macrophage subpopulations on these interactions and the consequences of such interactions will be studied.

The regulation of normal and paraprotein immunoglobulin production in multiple myeloma will be studied with a focus on the role of the monocyte/macrophage in this interaction. In vitro results will be correlated with clinical findings. Using the myeloma idiotype as a prototype tumor-specific antigen the ability of anti-idiotype (tumor-specific) antibody to 'arm' normal macrophages for specific tumor cell binding will also be assessed. A further understanding of the mechanisms involved in macrophage recognition and alteration of cell surfaces should provide insight leading to new approaches for harnessing the macrophage's ability to detect and alter tumor cells. These studies should also provide insight into the nature of activation and regulation of malignant (myeloma) cells and normal immunoglobulin-producing cells in a malignant lymphoproliferative disorder.

340. FUNCTIONS OF MACROPHAGE SUBPOPULATIONS IN IMMUNE AND NONIMMUNE MECHANISMS

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OBJECTIVE: The project aims to elucidate the developmental sequence of subsets of mph with antigen-presentations and anti-tumor effector functions.

APPROACH: Macrophages at various stages of development will be obtained by cell size fractionation, treatment with anti-Ia sera or anti-macrophage monoclonal antibodies, and by cultivation of bone marrow precursors in vitro. They will be assessed for Ia-dependent antigen-presenting activity in the induction of delayed-type hypersensitivity and proliferative, humoral and cytotoxic responses to tumor, minor histocompatibility and synthetic peptide antigens as well as anti-tumor effector mechanisms after activation. These functions will be correlated with changes in cell surface components detectable with monoclonal antibodies.

PROGRESS: The antigen-presenting cell in the peritoneal cavity was found to be a small, Ia positive macrophage whereas anti-tumor activity was associated with large macrophages. Macrophages cultivated from bone marrow cells have different contents of Ia positive cells and antigen-presenting function depending on the source of colony-stimulating factor. The active cell from these cultures is also a small macrophage.

341. THE ROLE OF MACROPHAGE SUBCLASSES IN TUMOR IMMUNITY

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The role of the macrophage effector cells in tumor immunity is to be studied in light of the functional heterogeneity of these cells. The cytotoxic activity (tumor growth inhibition and tumor lysis) of the light density population of macrophages from normal or stimulated animals will be compared to the activities of activated macrophages and subpopulations of activated macrophages obtained from animals injected with C. parvum. These comparisons will include selective recognition for neoplastic cells and enzymatic activities associated with macrophage activation. The mode of action of the stimulated macrophage subpopulations that inhibit or augment tumor growth will be elucidated in respect to interaction kinetics, effects on tumor cell membranes and tumor cell metabolism. In addition, the in vitro tumor-growth inhibitory activity of macrophage subpopulations will be assessed in vivo.

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342. NATURAL AND ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY

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Macrophages may be the primary anti-neoplastic surveillance cells, especially the alveolar macrophages, which may be the most important defense mechanism protecting the lung from neoplastic growth as well as invading microbes. Conventional and specific pathogen-free (SPF) newborn colostrum-fed piglets have high numbers of neutrophils infiltrating the lung within 12 hrs after birth which are replaced with large numbers of alveolar macrophages within a week. However, the germfree colostrum-deprived immunologically 'virgin' piglets have absolutely no alveolar macrophages. Therefore, the germfree piglets present a unique opportunity to us to investigate: 1) ontogenic development and differentiation of alveolar macrophages in the controlled environment starting from zero level; 2) characterization of the nature of effector cells for natural cytotoxicity, antibody-dependent cellular cytotoxicity (ADCC) and complement (C3)-dependent cellular cytotoxicity (C3DCC) against various targets (syngeneic, allogenic and xenogeneic targets; tumor cells or transformed cells vs. untransformed cells); and 3) factors influencing the development and differentiation of alveolar macrophages and factors affecting their effector functions against target cells. Investigations on ontogenic development, differentiation of alveolar macrophages and regulatory mechanisms of their cytolytic and cytostatic effector functions may eventually lead to manipulation or control of alveolar macrophages against invading cells to confer on the host a better survival advantage.

343. THE INTERACTION OF MACROPHAGES AND TUMOR CELLS IN VIVO

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Macrophages can be stimulated to be specifically or nonspecifically cytotoxic to tumor cells in vitro, and cytotoxic macrophages accumulate in response to growing tumors. Nevertheless, most syngeneic tumors, even those infiltrated with macrophages, grow progressively and kill their hosts. I have found that the ip injection of the weakly immunogenic ascitic leukemias EL4 and L1210 into syngeneic mice elicits the accumulation of large numbers of macrophages in the peritoneal cavity, spleen and lymph nodes and that the same reaction is produced by supernatant fluid from frozen-thawed (FT) tumor cells. Despite the magnitude and systemic nature of this response, these tumors are rapidly lethal. I will attempt to demonstrate that the failure of these macrophages to control the growth of the tumors in vivo is due to a cellular or soluble inhibitory factor. The experimental approach will be to test the capacity of materials derived from the mouse (lymphocytes, serum, ascitic fluid) and from the tumor cells (culture supernatant, lysates) to inhibit macrophage-mediated cytotoxicity in vitro. The cytotoxic macrophages will be obtained from mice injected with FT tumor cell preparations or Corynebacterium parvum. I will study the specificity and chemical composition of any inhibitory factor that is identified. In addition, I will investigate the accumulation of macrophages in response to FT tumor cell preparations to determine if this is a specific response to tumor antigens or a non-specific response to a substance common to tumor cells.

344. EFFECT OF DIFFUSIBLE MACROPHAGE PRODUCTS ON NORMAL AND MNU-TREATED RAT BLADDER ORGAN CULTURES

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The majority of reports stress the cytostatic and cytotoxic effects of mononuclear phagocytes on tumor cells. It is also known that macrophages exert profound lymphoregulatory influences. There is, however, some evidence to suggest that their regulatory activities are not confined to the lymphoid system. Enhancement of growth of fibroblasts, tumor cell lines and tumor explants by macrophages has been observed in certain experimental systems. We have chosen to study this phenomenon further using the bladder organ culture system of Hodges. Preliminary experiments on bladder explants, from normal and in vivo MNU-treated rats, and cultured in the presence of oil-induced rat splenic macrophages, have shown that in both groups there is a considerable enhancement of epithelial growth. Experiments are in progress to determine whether the observed effects can be mimicked by mercaptoethanol and whether other cell types can also exert a similar effect.

345. TUMOR CELL KILLING BY A SOLUBLE MACROPHAGE PRODUCT

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There is considerable in vitro evidence in the literature which suggests that macrophages from animals with chronic infections or which have been treated with immunomodulating agents play an important role in prevention of tumor development or in resistance to existing tumors. However, the mechanisms by which activated macrophages recognize and kill target cells are not yet clearly understood. This project is designed to examine the effect of soluble macrophage products on the expression of their cytotoxicity in animal systems. The role of soluble factor(s) in the expression of human monocyte-macrophage-mediated lysis of malignant cells will also be evaluated. We also hope to determine if monocyte-macrophages from cancer-bearing patients contain or release these products. It is hoped that these studies will provide further information on the mechanisms of monocyte-macrophage-mediated cytotoxicity.

346. MECHANISM OF THE ANTITUMOR ACTIVITY OF MACROPHAGES ACTIVATED BY CORYNEBACTERIUM PARVUM

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OBJECTIVE: The objective of this investigation is to study the mechanism of activation of macrophages exposed to CP, and the in vitro and in vivo antitumor activity of activated macrophages.

APPROACH: The approach is as follows: 1) a better morphological and functional definition of activated macrophages, using cytochemical reactions to characterize subpopulations with different activities, and using cellular fusion to demonstrate membrane modifications induced by activation; 2) gradient separation of macrophage subpopulations to test the antitumor activity of separated macrophages on tumor cell cultures in vitro and on experimental tumors in vivo; 3) evaluation of a higher production of CSA by activated than by non-activated macrophages.

PROGRESS: Preliminary results have been obtained concerning cellular modifications induced by CP on mouse peritoneal exudate cells (PEC). Two sets of CDI mice (age - 30 days, mean body weight - 1.6 g) were inoculated i.p. with 0.1 ml CP (Wellcome). After inoculation, the first set of mice was killed daily from day 1 to day 23, and the following evaluations were performed: 1) evaluation of the number of macrophages, lymphocytes and polymorphonucleated cells among PEC in unfixed smears; May-Grunwald Giemsa staining and ANAE reaction in cytocentrifuged fixed smears; 2) evaluation of spleen and liver fresh weight and fixation in formalin. The second set of mice was inoculated daily i.p. with 0.5 mg/kg body weight of Vincristine, and after 14 hours the mice were killed and the stathmokinetic indices of macrophages were determined on PEC cytocentrifuged, May-Grunwald Giemsa stained smears. The results obtained by now show that the macrophage activation induced by CP (expressed by the increase of peritoneal macrophage number, the increase of macrophage stathmokinetic index, and the increase of spleen and liver weight) reaches the maximum about day 8, and then it is maintained at lower levels, with little increase and decrease. Moreover, peritoneal macrophages seem to be recruited from spleen and liver particularly on days 3, 13 and 23, when spleen and liver weight decreases.

347. LYSOSOMES IN MACROPHAGE ANTITUMOR AND ANTIVIRAL ACTIVITY

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OBJECTIVE: The project will investigate the biological significance and role of lysosomes in macrophage resistance to tumor cells and viruses by using an animal model (the beige mouse) with lysosomal deficiencies.

APPROACH: The susceptibility of the beige mouse (C57BL/6Jbg/bg) to Lewis lung carcinoma and to herpes simplex virus (HSV) infection will be determined, relative to that of wild-type control. Tumor cell cytolysis by beige mouse macrophages will be determined using a 125IUdR release assay. Lysosomal transfer (after staining with acridine orange) and cytolysis of tumor cells will also be evaluated by a morphologic assay. Characterization of macrophage populations from the beige mouse will be done as an adjunct to these investigations.

The antitumor activity of beige macrophages will be compared with antiviral activity of these cells, using HSV as a model. Activated beige macrophages will be evaluated for their ability to reduce HSV plaques and virus yield in infected cells. Kinetics of clearance of viral antigens and DNA in macrophages, and possible latency of viral DNA, will be evaluated.

Finally, treatments which have been reported to reverse the Chediak-Higashi syndrome (the genetic disease of which the beige mouse is a homologue) will be tested for their effects on macrophage antitumor activity.

348. ROLE OF MACROPHAGE ARGINASE IN TUMOR IMMUNITY

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I propose to target liposomes containing iron oxide particles (magnetosomes) and a drug to defined areas in rats by the use of an external magnetic field. The encapsulation of iron oxide particles in liposomes (magnetosomes) in high yield is made possible by a new technique developed in this laboratory (Szoka and Papahadjopoulos, Proc. Nat. Acad. Sci. USA, 75, 4194-4198 (1978). Furthermore, magnetosomes can be prepared in defined size ranges by extrusion through polycarbonate membranes, an important parameter for in vivo drug delivery. The magnetosomes will be examined in an in vitro analog of the circulatory system to determine the effects of the intensity of the magnetic field and flow rate through the system on the localization of magnetosomes. The location of the magnetosomes will be followed by including both a radiolabelled phospholipid in the bilayer and a radiolabelled soluble marker in the aqueous space. The effect of size, surface charge, and lipid composition on the localization in both buffered saline and plasma will also be examined. For the in vivo system, both rats and mice will be used to determine the distribution of intravenous, intraperitoneal, and intrarterial injected magnetosomes. The effect of a directed external magnetic field on the localization of magnetosomes into different regions of the body will be established. Strategies to increase localized blood flow such as heating or vasodilators as a means of increasing the through put of magnetosomes will be investigated. Finally, I will examine the delivery and antitumor effect of magnetosome encapsulated drugs against the Lewis lung carcinoma and the B-16 melanoma tumor systems. In these models, the mode of injection of the malignant cells can lead to a localized tumor that after a characteristic time period can metastasize. Thus, they are excellent models to investigate the efficiency of magnetosomes as a targeted drug delivery system.

349. CHARACTERIZATION OF FACTORS CAUSING INHIBITION OF MACROPHAGE FUNCTION OR INFLAMMATORY RESPONSES

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SCOPE: Conduct research designed to functionally characterize factors produced by and/or induced by tumors, which impair macrophage function and/or inflammatory responses in vivo and in vitro.

GOAL: This project falls under Objective 3, Approaches 3 and 4 and Objective 6, Approaches 1 and 4 of the National Cancer Plan.

Utilization of Project: Use is as an essential part of the immunologic studies of neoplastic disease.

350. STUDIES OF A TUMOR FACTOR THAT AFFECTS MACROPHAGES

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Tumors may have the capacity to subvert host defense mechanisms and thus escape from destruction by the host. Recent studies indicate that this may in part be mediated by soluble factors produced by the tumor cells. Several mouse tumors that we have examined produce a low molecular weight factor which affects macrophages resulting in the modulation of their properties. This research proposal seeks to undertake the functional and biochemical characterization of this factor. The factor(s) will be purified and tested for its effects on macrophages, polymorphonuclear leukocytes and properties of lymphocytes such as proliferative response to mitogens, proliferative response in mixed leukocyte culture reaction, generation of cytotoxic T lymphocytes in mixed leukocyte culture, T cell mediated cytotoxicity and immune response. The macrophage modulating factors produced by different tumors will be examined biochemically and functionally to determine if they are identical in nature. Also, tumor bearing hosts will be examined for the presence of this factor in circulation. In addition, purified factor will be tested for anti-inflammatory effect and effects on antibody response and cell mediated immunity in vivo. The purified factor will be conjugated to carriers, the conjugates will be used as immunogens and attempts to raise antiserum against the factor in rabbits will be made so that the antiserum can be used for the estimation as well as neutralization of the factor. Finally, the role of the tumor factor, if any, on tumor-host relation will be examined by studying the effects of independent injections of purified tumor factor and antibody against the factor on the mean survival time of tumor bearing hosts. These in vitro and in vivo studies should provide information on whether and how the tumor factor subverts host defense mechanisms and facilitates the spread of tumor.

351. CELL INTERACTIONS INITIATING SYNGENEIC IMMUNITY

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Macrophages provide an essential component of the host's immune response to neoplasia. In vivo, macrophages participate as effector cells as evidenced by their presence within tumor foci and their capacity to transfer tumor resistance to syngeneic recipients. Following interactions with lymphocytes and/or soluble products secreted by these cells, macrophages become cytotoxic for tumor cells or, at least, inhibit their growth.

Recent investigations from this laboratory have demonstrated that two powerful immunostimulants, bacterial lipopolysaccharides (LPS) and bacillus Calmette-Guerin (BCG), synergistically inhibit growth of ascites tumors in syngeneic mice. Our preliminary studies suggest that BCG infection activates macrophages extensively while LPS augments the tumoricidal capacity of these cells.

This proposal is designed to investigate the effects of LPS on BCG macrophages in vitro. Using cytochemical and autoradiographic techniques, we will quantitatively determine membrane binding, internalization and intracytoplasmic localization of LPS in macrophages. Other aspects of LPS-macrophage interactions such as phagosome formation, lysosome fusion and extracellular release of lysosomal enzymes will also be explored. Attempts will be made to correlate LPS-induced alterations with increased tumoricidal activity. The effects of drugs which increase or decrease lysosome stability will be analyzed in detail.

By delineating events which characterize LPS interactions at the cellular level, these studies are expected to provide valuable insight and substantial new knowledge regarding the mechanisms of macrophage-mediated cytotoxicity. Furthermore, clinical administration of BCG and LPS, using appropriate therapeutic regimens outlined in this proposal, offers a promising approach for potentiating the host's immunity to autochthonous tumors.

352. MACROPHAGE HETEROGENEITY IN TUMOR IMMUNITY

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This research proposal continues the investigation of macrophage (M0) heterogeneity as it relates to host defense mechanisms against tumors. Peritoneal M0 from non-immune, Corynebacterium parvum treated, and Moloney sarcoma tumor bearing rats and tumor-derived M0 will be separated into subpopulations by density gradient, centrifugal elutriation, and flow cytometry procedures. Cells in each subpopulation will be examined for effector function in vivo using adoptive transfers and in vitro using cytolytic and cytostatic assay systems. M0 in each subpopulation will also be tested for their ability to interact specifically and non-specifically with tumor cells in the presence or absence of antibody. An important aspect of this proposal will be the evaluation of suppressor vs. enhancer function of various subpopulations of M0. In this study such systems as mitogen-induced blastogenesis, mixed lymphocyte reactions and mixed lymphocyte tumor reactions will be evaluated. Suppressor vs. enhancer function of M0 subpopulations will also be evaluated in vivo using a Winn type assay. Helper activity will be studied during antibody synthesis in vitro in a clonal and polyclonal antibody response. Cells in each subpopulation will be characterized by morphologic examination and by measurement of phagocytic capability, Fc, and C3b receptor activity and enzyme levels. A special effort will be made to relate such parameters with M0 activation in order to identify easily demonstrable markers of such activity in M0 subsets.

353. INDUCTION AND COMPARATIVE STUDY OF MACROPHAGE-RESISTANT AND MACROPHAGEENSITIVE TUMORS

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OBJECTIVE: To further elucidate the role of macrophages in the resistance to tumors.

APPROACH: A previous study has shown that the incidence of sarcomas following a single injection of methylcholanthrene is reduced in mice given repeated injections of C. parvum starting prior to injection of the carcinogen. Two tumors which developed under these conditions have been found to be insensitive to treatment with C. parvum in vivo and one is also insensitive to the cytotoxic action of activated macrophages in vitro. It is proposed to delineate the conditions under which macrophage-insensitive tumors develop and to undertake a comparative study of the behavior of macrophage-insensitive and macrophage-sensitive tumors in vivo (using normal and athymic hosts) and in vitro, with the object of elucidating further the role of macrophages in cancer homeostasis, the mechanism by which macrophage-insensitive tumor cells arise, the capacity of macrophages to recognize neoplastic cells and the nature of markers on which this recognition depends.

PROGRESS: Many tumors have been induced in mice of different strains under a variety of experimental conditions, and their response to macrophages in vitro and to macrophage stimulating agents in vivo is currently being studied. We are also studying the histological features of early developing primary tumors.

354. THE IMMUNOGENICITY OF TUMOR-MACROPHAGE HYBRIDS

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DBA2 skin grafts placed on BALB/c mice are uniformly rejected although the two strains are identical H-2d. Despite this we have found two situations in which organ grafts from DBA2 to BALB were accepted. First, thyroid grafts transferred from DNA2 to BALB underwent a period infiltration, which peaked at three weeks and then cleared up by 6 weeks. In 75% of the transplants a functioning thyroid graft was retained as indicated by 125I uptake and a characteristic histology. At this time the transplantation of B6D2 thyroids or skin grafts was followed by rejection of the skin grafts at the expected time (10-12 days). Despite the rejection of the B2D2 grafts, the DBA2 thyroid grafts applied earlier were obtained.

Another situation where unexpected tolerance of DBA2 grafts was obtained was found in radiation chimeras after destruction of the chimera. BALB/c mice were first made chimeras by injecting DNA2 bone marrow after 750 gamma,gamma-radiation. DBA2 skin grafts were applied and accepted. At this point spleen cells from BALB/c mice which had rejected DNA2 skin grafts were injected. Although the chimera was destroyed by this injection, the DBA2 skin graft was retained.

Furthermore, the rejection of a new B2D2 skin graft did not result in a rejection of the DBA2 graft.

In both situations described above the rejection of a hybrid graft containing both major and minor antigenic differences with regard to the host did not result in rejection of a graft containing only minor antigens. We believe that the reason for this failure may be that many minor antigens are not expressed on every cell. Stated another way, the minor antigens found on stimulating cells may not be found on non-stimulating cells.

355. MACROPHAGE FUNCTIONS IN TUMORIGENESIS

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The objective of this research program is to gain a better understanding of macrophages and their responses to tumors. An established cell line of mouse peritoneal macrophages (IC-21 macrophages), which possess cell-mediated immune reactivity, will serve as a macrophage model but relevant experiments will be repeated with macrophages from mice. The program is divided into two separate but related projects, the first of which will further define and characterize the immunoglobulin class-specific Fc receptors on macrophages and will relate their presence to events that occur as a result of recognition at those sites.

The second project will utilize information obtained in the first project and will employ syngeneic tumor lines to relate specific anti-tumor responses of macrophages to specific cells. This project will exploit the ability to separate peritoneal macrophages into density-dependent subpopulations of cells that are functionally distinct. I will examine the ability of the subpopulations to be rendered anti-tumor effector cells by both immunologically specific and nonspecific means, and will attempt to relate any alterations in macrophage functions to specific subpopulations of cells where subtle yet significant differences can be detected.

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356. CHANGE IN RETICULOENDOTHELIAL PHAGOCYTOSIS DURING CHEMICAL CARCINOGENESIS

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OBJECTIVE: To investigate change in hepatic and splenic phagocytosis of radiochromium-labeled xenogeneic red cells in mice during the latent period of chemical carcinogenesis and test the relationship of these changes to subsequent development of tumors.

APPROACH: Inbred mice, injected with methylcholanthrene, are subsequently injected with 51Cr labeled rat red cells, and 24 hours later they are either killed and hepatic and splenic uptake of radiolabel is determined, or they are surgically splenectomized for assay of splenic phagocytosis and observed for development of tumors. These tests are done on groups of controls and carcinogen-treated mice at intervals ranging from 2 to 15 weeks after administration of methylcholanthrene. The correlation between changes in phagocytosis with tumor appearance is tested in mice permitted to survive.

PROGRESS: Confirming and expanding previous findings, results to date suggest that impaired splenic phagocytosis is the most consistent change during the latent period of chemical carcinogenesis, analogous to findings obtained with transplanted mouse tumors and spontaneous tumors in mice.

357. RADIATION AND THE MONONUCLEAR PHAGOCYTE RESPONSE TO CANCER

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The fluorescence-activated cell sorter will be used to quantify the number of lymphocytes and mononuclear phagocytes within several murine tumors. Utilizing specific cell surface markers for lymphocytes and macrophages, those cells will be sorted for subsequent functional analysis.

The murine tumors selected for this study vary in their ability to arouse a detectable host defense and represent different histological classes. The EMT6 mammary tumor is immunogenic; the KHT fibrosarcoma is not. While these two tumors have a high macrophage content, the B16 melanoma does not. All three tumors culture well in vitro. In contrast, the Lewis lung carcinoma and 6C3HED lymphosarcoma represent tumors with low and high macrophage content respectively but, while tumor cells survive poorly in vitro, the macrophages from them have a high plating efficiency.

We plan to study the secretion of the macrophage activating factor (MAF) tumor derived lymphocytes. This factor is required for rendering macrophages tumoricidal. The clonogenic and tumoricidal activity of tumor derived macrophages and the ability of the tumor cells to elaborate macrophage growth factor (MGF), a factor necessary for macrophages to proliferate, will also be determined. With this knowledge, we will be able to define in a more realistic fashion the cellular interactions between lymphocytes, macrophages, and tumor cells within progressing and regressing tumors.

Utilizing the tests, we will investigate the effect of single and fractionated tumor irradiation on the host defense elements. Mice bearing tumors will be irradiated while the tumor is in log growth and each parameter will be evaluated in animals bearing progressing and regressing tumors. Results from this study will reveal important information on why some tumors progress and others regress by defining the number of host defense cells relative to tumor cells and their tumoricidal state.

358. INFLAMMATORY RESPONSE AND PHAGOCYTIC FUNCTION IN IMMUNODEFICIENCY

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This is part of a broader project. A summary of this subproject is not available.

359. ROLE OF MONONUCLEAR PHAGOCYTES IN TUMOR IMMUNITY (MICE)

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This is part of a broader project. A summary of this subproject is not available.

360. LDH-VIRUS GROWTH AND EFFECT ON MACROPHAGE FUNCTIONS

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The ultimate objective of the proposed investigation is to elucidate the mechanism by which the 'non-cytopathogenic' lactate dehydrogenase-elevating virus (LDV) only replicates in macrophages and similar types of cells and how virus replication operates within the framework of the regulatory processes of the host cell. The underlying question is how LDV affects various macrophage functions as exemplified by its inhibitor of blood enzyme clearance and its possible effect on the immune systems of the host. Related is the question of why only a relatively small proportion of macrophages in primary cultures can be productively infected with LDV. This has been recently discovered by the use of an autoradiographic method developed by us. We will approach all these questions by monitoring various macrophage functions, Fc and C3 receptors, and LDV replication in individual cells of macrophage populations at various times after placing the cells in culture. Macrophages will be propagated in mouse L-cell conditioned medium, which stimulates the replication of these cells in vitro. Antibodies to LDV have been isolated from chronically infected mice and conjugated with fluorescein isothiocyante. It will be used in conjunction with above methods to assess LDV replication in individual cells of infected cultures or mouse tissues. We will also use anti-LDV antibodies to develop an in vitro procedure for its titration. Other studies concern the question of whether the transient high level production of LDV in macrophages and the following chronic persistent infection are related to interferon production. The question of whether LDV enhances tumor induction by RNA tumor viruses will be investigated by studying the replication of LDV and mouse RNA tumor viruses in long-term macrophage or lung-spleen cultures. Studies on the physical-chemical characteristics of LDV and the method of replication will be continued. A search will be made in other species, including man, for viruses of a type similar to LDV. Human blood samples will be screened for elevated enzyme plasma levels and suspected samples will be analyzed for virus by inoculation into human macrophage cultures.

BIBLIOGRAPHIC REFERENCES: Brinton, M.A. and P.G.W. Plagemann. 1979. Actinomycin D Cytotoxicity for Mouse Peritoneal Macrophages and Effect on Lactate Dehydrogenase Elevating Virus Replication. Int. J. Virol. 12:349-356.

361. AGE-DEPENDENT ALTERATIONS OF ADHERENT CELL FUNCTION

Stohlman, S. A., Frelinger, J. A., University of Southern California, School of Medicine, Dept. of Neurology/Microbiology, *2025 Zonal Ave., Los Angeles, California*, 90033, United States of America

We will study the role of macrophages, which appear to act as primary effector cells in conferring protection from lethal viral-induced central nervous system disease. Preliminary studies show that SJL mice exhibit an age-dependent resistance to central nervous system infection due to the neurotropic JHM strain of mouse hepatitis virus. Resistance can be transferred from old (12 week) resistant mice to young (6 week) susceptible animals with either splenic or peritoneal macrophages taken from animals which exhibit no evidence of immunity to mouse hepatitis virus. Macrophages from 2 and 12 week old animals will be compared to determine if additional age-dependent changes in macrophage functions can be found. The peritoneal exudate cells from the two age groups will be compared for the number of total macrophages; the level of 'activation'; the numbers of Ia antigen, Fc, and C3 receptor bearing cells; and the ability to function as phagocytes, as ADCC effectors, and as T and B cell helpers. In addition, we will examine possible genetic restrictions conferred upon these cells in their role as primary effector cells and the possible interactions with other cells of the immune system.

362. MACROPHAGE ROLE IN IMMUNITY TO MURINE LEUKEMIA VIRUS

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The role of the macrophage in the afferent arm of the immune response of mice to endogenous, ecotropic murine leukemia virus (MuLV) will be examined. Several lines of evidence indicate that the MuLV-specific immune response may be an important host defense in the regulation of virus proliferation and hence acts as a host defense against malignancy. The macrophage plays a key role in the initiation of many immune responses. Its function, however, in the immune response to MuLV has not been characterized.

This macrophage function will be defined by exposing purified macrophage populations to MuLV or MuLV antigens in vitro and subsequently assessing their ability to initiate a specific immune response in several different systems. These experiments will include an in vitro T-cell proliferation assay, an in vitro assay for MuLV-specific T-helper cell activity, and in vivo adoptive transfer techniques.

The examination of macrophage function will be applied to two experimental systems which the principal investigator has previously developed. The first is a model for studying the influence of the major histocompatibility complex (MHC, H-2) upon susceptibility to virus infection. There is evidence that this influence may reflect the operation of an immune response (Ir) gene, possibly expressed through the macrophage. The second system involves the phenomenon of neonatal susceptibility of mice to MuLV infection, which may be due to a lack of functionally mature macrophages.

363. MACROPHAGE EXTRINSIC ACTIVITY VS VIRUSES

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Major emphasis will continue to be placed on defining macrophage heterogeneity among peritoneal macrophage subpopulations separated on the basis of density. We have achieved basic information with C. parvum macrophages, and this information will now be obtained for resident and thioglycollate elicited macrophages. In addition to the current assays for ectoenzyme phenotypes and antitumor activity, we plan to determine permissiveness of the subpopulations for HSV. Our preliminary results with total peritoneal macrophage populations revealed that resident and C. parvum macrophages are almost completely resistant to HSV infection, while there was erratic growth in the thioglycollate macrophages. Determination of susceptibility of separated subpopulations may show that certain populations have enhanced susceptibility while others are as resistant as the resident macrophage population.

Our research currently is focussed on separation on Ficoll discontinuous density gradients. It has taken considerably longer than expected to get the Beckman elutriation centrifugation system in Dr. Miller's laboratory functioning well on a routine basis. We expect to perform experiments with the elutriator to confirm that results similar to Ficoll separation are obtained and then move completely to the elutriator. In addition, we plan more studies of the separated subpopulations using the FACS cell sorter analysis capability.

The second thrust of the research will be directed at macrophage cell lines, delineating biochemical markers and permissiveness for virus infection. The continuing work with J774.1, WEHI-3 and PU5-1.8 mouse macrophage cell lines has been detailed above. We are now initiating studies with the immature human macrophage cell line, U937. This cell line, upon treatment with lymphokine, 'activates' in regard to ADCC activity and phagocytic activity. We are documenting the changes that occur in ectoenzyme phenotypes and permissiveness for HSV during this progression. Laura Googin will be responsible for this latter project.

364. IMMUNOREGULATORY EFFECTS OF BACTERIAL SUBSTANCES

Mishell, R. I., Fairchild, S., Rostam Abadi, H., University of California, Berkeley Campus, School of Letters & Science, Dept. of Microbiology & Immunology, *Berkeley, California*, 94720, United States of America

Studies will be continued on bacterial substances which induce murine macrophages to synthesize and release immunoregulatory mediators. The bacterial substances include lipopolysaccharides, peptidoglycans, muramyl dipeptide, lipoteichoic acids and bacterial membrane proteins. One aspect of the research is to indentify membrane receptors in murine macrophages which specifically bind the bacterial inducers. We are also investigating the mediators produced by macrophages following induction. Some of these are detected by their ability to block the immunosuppressive effects of glucocorticosteroid hormones. These are being purified and their effects on various types of cells is being investigated.

BIBLIOGRAPHIC REFERENCES: Mishell, R. I. and Y. U. Chen. 1980. Activation of B lymphocytes and macrophages by peptidoglycan subunits and muramyl dipeptide. Fed. Proc. 39: 913. Slomich, M., S. M. Shiigi, E. Kwan and R. I. Mishell. 1980. Biology of accessory cells required for antigen-specific T lymphocyte proliferation. Fed. Proc. 39:912.

365. INTERACTION BETWEEN HOST PHAGOCYTES AND LEGIONELLA PNEUMOPHILA

Horwitz, M. A., Silverstein, S. C., Rockefeller University, Cellular Physiology & Immunology Lab., *66th & York Ave., New York, New York*, 10021, United States of America

We plan to use in vitro techniques to study the interaction between Legionella pneumophila, the agent of Legionnaires' disease, and host cellular and humoral immune systems. We shall use virulent chicken embryo derived cultures of L. pneumophila, human blood monocytes, human skin-window derived macrophages, and guinea pig peritoneal macrophages. Preliminary studies indicate that L. pneumophila is resistant to the bactericidal effects of serum, antibody, and human polymorphonuclear leukocytes, and that under physiologic conditions, the bacteria require the presence of human monocytes for growth: growth is probably intracellular. We plan to determine (1) if L. pneumophila is an obligate intracellular parasite under physiologic conditions, (2) if so, the mechanism by which L. pneumophila survives intracellularly (e.g., by inhibiting phagosome-lysosome fusion, by resisting toxic oxygen intermediates, by escaping the phagosome into the cytoplasm, etc.), (3) if human monocytes and guinea pig macrophages activated by lymphokines in vivo or in vitro are capable of killing the bacterium, and (4) the mechanism by which human and animal macrophages kill the bacterium and the role of toxic oxygen metabolites in this cytotoxicity. We believe that the in vitro model that we have developed for studying the interaction of L. pneumophila and human phagocytes will prove to be an excellent one for general studies of host-parasite relationships and macrophage cytotoxic effector mechanisms.

366. MIGRATION AND PHAGOCYTOSIS IN VITRO

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OBJECTIVE: Functional assay of granulocytes, monocytes and cancer cells.

APPROACH: To develop new, simple and precise methods of assaying chemotaxis and chemokinesis of professional phagocytes, as well as cancer cells. Similarly, to modify existing assays for phagocytosis and microbial killing by phagocytes (3H-thymidine-labelled bacteria; fluorochrome-labelled bacteria and phagocytes analysed with flow cytometry).

PROGRESS: A new cell migration chamber has been constructed, allowing cells to migrate through Millipore filter heat-sealed to the opening of a plastic container. Quantitative recovery of cells migrated through the filter has been obtained, permitting electronic counting, analysis by flow cytometry or routine cytological investigation, and further functional studies.

C. REGULATION AND OTHER ROLES OF MONOCYTES AND MACROPHAGES

367. THE IMMUNOGENETICS OF MACROPHAGE SUPPRESSION IN MAN

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We propose to study in vitro a human suppressor monocyte/macrophage system. Our data which provide the background for this proposal show that suppressor monocyte/macrophages can be elicited by in vitro culturing of normal, peripheral blood mononuclear cells. These suppressor cells can be purified by Ficoll density gradient centrifugation. The cultured monocyte/macrophages are capable of suppressing allogeneic stimulation, generation of cytotoxic cells and mitogenic stimulation. We are able to extract a soluble factor from such cells which itself can suppress the mixed lymphocyte reaction. Using a panel of fully HLA-A, -B, -C and -DR typed normal donors, our objective is to: a) study the importance of HLA-DR (Ia) specificities at the level of suppressor monocyte/macrophages and at the level of suppressor soluble factors as each interacts with HLA-DR typed responder cells stimulated by alloantigen or by pokeweed mitogen (PWM). We shall measure alloantigen stimulation by 3H-thymidine incorporation and mitogenic stimulation by immunoglobulin production, using a radioimmunoassay. The suppressor values obtained in checkerboard-type cell combinations will be used to a) evaluate clustering of suppression values in relation to the number of HLA-DR specificities shared, i.e., two, one or none between each suppressor and responder cell pair; b) characterize the soluble factor from physical and immunochemical points of view; this will include: stability, molecular weight, solid phase immunoadsorptions by the use of Sepharose coupled xenoantisera directed against: human immunoglobulin, human beta 2 microglobulin, human p29,34 (Ia); c) establish the antigenic uniqueness of suppressor monocyte/macrophages from the point of view of cell surface differentiation antigens by preparing xenoantisera to these cells and by rendering them specific through successive adsorptions with Sepharose-pooled human serum, autologous red blood cells, autologous T cells, Daudi cells and eventual adsorption and elution from chemically fixed autologous suppressor monocyte/macrophage cells.

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368. REGULATION OF GRANULOCYTE AND MACROPHAGE PRODUCTION

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This proposal is directed towards: 1) understanding the biochemistry, mechanism of action and physiological role of a subclass of colony stimulating factors (CSFs) sharing identity with macrophage growth factor, and 2) using purified CSF and its specific antibody as probes to investigate the early events in hemopoiesis. Working with murine and human material, attempts will be made as often as possible to apply murine findings to the human system.

Further work on the biochemistry of L cell and human urinary CSF, including investigation of the nature of the subunits, amino acid and carbohydrate analyses and the amino acid sequence determination, will be continued. Studies on the mechanism of action will be carried out using biologically active 125I-CSF. The nature and distribution of CSF binding cells in normal and leukemic murine and human cell populations will be examined. The mechanism of CSF interaction with target cells, the relationship between target cell binding and response and the characteristics of the CSF 'receptor' will be investigated. Parameters relating to blood cell differentiation and the mononuclear phagocytic system will be monitored in mice following injection of purified L cell CSF and its specific antibody to determine the physiological role of CSF.

Highly specific antibody to both L cell and human urinary CSF will be prepared. These antibodies will be used to examine the nature and distribution of CSF releasing cells and to examine the genetic control of human CSF production using human-mouse cell hybrids. Studies of the mechanism by which pluripotent stem cells give rise to the most immature CSF-responsive cells will be commenced.

369. DIFFERENTIATION OF GRANULOCYTES AND MONOCYTES

Metcalf, D., Burgess, A. W., Watt, S. M., Nicola, N. A., Cooper, P., Walter & Eliza Hall Inst. for Medical Research, *c/o Royal Melbourne Hospital, Melbourne, Victoria 3050, Australia*

Molecular aspects of the regulation of granulocyte-macrophage production from colony-forming cells form the basis of this project. Hemopoietic regulators are produced in conditioned media and purified using column chromatography and electrophoresis. The molecular properties of GM-CSF and CFS's specific for other cell lineages are studied in detail. Separation of progenitor cells and immature myeloid cells is achieved using the fluorescence-activated cell sorter for quantitative analysis of lectin binding. Molecular changes induced by GM-CSF in progenitor cells, mature neutrophils and a myelomonocytic leukemia, WEHI-3B, are being analyzed using two-dimensional electrophoresis.

BIBLIOGRAPHIC REFERENCES: Metcalf, D., Russell, S. H. M. and Burgess, A. W. Production of hemopoietic stimulating factors by pokeweed mitogen-stimulated spleen cells. Transplant. Proc. 10, 591-594 (1978).

370. MACROPHAGE REGULATION OF ANTITUMOR IMMUNOCYTOTOXICITY

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The immunoregulatory functions of macrophages in the afferent phase of cell mediated immune (CMI) responses has received little attention and the mechanism by which these cells function in the induction of cell mediated cytotoxic responses is unknown. While it is clear that this cell is important in regulating the cellular events of antibody formation, the role of the macrophage in the induction of thymus derived (T) cells is less clear. The macrophage has a critical, although yet undefined, role in the induction of classical skin test cell mediated immunity (CMI) in vivo and various models of CMI in vitro. The mitogenic and allogeneic activation of T cells has been shown to be dependent on macrophages but the exact mechanism by which these cells function in the above phenomena is unknown. We propose to determine the mechanism by which macrophages regulate the afferent phase of the generation of antitumor cell mediated cytotoxicity (CMC). Preliminary data suggest that macrophages act as: 1) stimulator cells, 2) accessory cells, 3) cells capable of presenting bound antigen to responder cells, and 4) cells possibly secreting soluble immunoregulatory products.

We have developed two in vitro model systems to explore the mechanisms of immunoregulation by macrophages in the afferent phase of the generation of CMC. Using murine spleen lymphocytes, we have developed one system to generate and measure CMC induced by allogeneic combinations of lymphocytes. In the other, we generate syngeneic, tumor specific CMC when C57BL6 spleen cells are stimulated by the syngeneic EL4 lymphoma cell. We have shown, on a preliminary basis, that macrophages are required for the generation of CMC in both systems. In addition, the presentation of macrophage bound alloantigen and syngeneic tumor specific antigen is quantitatively more efficient in inducing CMC than equal numbers of free allogeneic lymphocytes or syngeneic tumor cells.

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371. DIETARY INHIBITION OF MACROPHAGE ANTITUMOR ACTIVITY

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The objectives of this project are two-fold: 1) to further characterize the inhibition of macrophage mediated tumoricidal activity and refractoriness to immunopotentiation in mice fed a hypercholesteremic diet and 2) to document the role of low density lipoprotein and cholesterol in the inhibition of macrophage mediated tumor cell cytotoxicity. Experiments have been designed to examine the discrepancy between the inhibition of tumoricidal activity in vivo and the ability to kill tumor cells in vitro. Previous experiments have established that macrophages are indeed activated in hypercholesteremic hosts but that they fail to accumulate at the tumor site in response to immunoadjuvants. The innate bactericidal activity of liver and spleen macrophages and the ability of specifically sensitized T lymphocytes to activate and mobilize blood monocytes to infected tissues will be determined both before and after administration of immunopotentiators. Several tumor systems and the synthetic immunopotentiator pyran will be used to establish the nonspecificity of the impairment and lack of effector function. The ability of the hypercholesteremic host to resist metastasis will be determined. Histopathological examination of tumor and lymphoid tissue will accompany all experiments. Finally, experiments are planned to examine the possibility that low density lipoprotein or cholesterol may modulate macrophage mediated tumoricidal activity at the level of the macrophage plasma membrane.

372. GROWTH REGULATION IN THE MONOCYTE-MACROPHAGE LINEAGE

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No summary has been provided to the Smithsonian Science Information Exchange.

373. MACROPHAGE SUPPRESSION AND NEOPLASIA

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No summary has been provided to the Smithsonian Science Information Exchange.

374. ADHERENT IMMUNOSUPPRESSIVE MONONUCLEAR CELLS FROM MICE BEARING PLASMACYTOMAS

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This is part of a broader project. A summary of this subproject is not available.

375. HUMAN MACROPHAGES AND OTHER CELLS IN CARCINOGENESIS

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The summary for this project is under entry no. 251.

376. MATURATION OF IMMUNOLOGICAL MEMORY PRECURSORS

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The objective of this study is to examine macrophage heterogeneity and genetic control of macrophage interactions with thymus dependent and thymus independent (B2 gamma and B1 gamma respectively) IgG B memory cells. The role of Ia positive and Ia negative macrophages will be studied in normal and CBA/N hybrid mice using specific antisera and thymus dependent and thymus independent forms of the trinitrophenyl hapten.

377. ROLE OF MACROPHAGE-LIKE CELLS IN ANTIBODY PRODUCTION

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Immune response (Ir) genes are expressed at the T-macrophage interaction, but due to genetic restrictions it is difficult to determine whether T cells themselves or just macrophages express Ir genes. Thus chimeric mice were constructed from irradiated (responder and non-responder) F1 animals repopulated with responder stem cells, or from the fusion of two eight-cell embryos (allophenic mice) of responder and non-responder origin. In both of these types of chimeras, T cells of non-responder genotype which had matured in the responder environment were phenotypically responders. However, macrophages did not change their phenotype in a responder environment, confirming that they are a prime locus of Ir gene action.

The nature of the product(s) controlled by Ir genes is not understood. These are expressed by macrophage-like cells, which also release complexes of Ia antigen and immunogen, coupled in a molecular entity termed 'GRF' (genetically related factor). Biochemical analysis of GRF has progressed to the stage where it is possible to visualize using labelled antigen or amino acid products in marcrophage supernatants which have been cleared by anti-Ia antisera, and thus presumably are partly GRF.

Characterization of the nature of antigen-presenting macrophages has proceeded by analyzing characteristics of these cells from peritoneum and spleen-adherent fractions. Dendritic cells that are adherent, light and lack Fc receptors (unlike most macrophages) have been purified from splenic-adherent fractions and shown to be potent inducers of mixed lymphocyte reaction, T cell proliferation induced by antigen and of helper cells.

The relationship of these cells to classical macrophages is still not clear and genetic restrictions have been reported at the interaction of T and B cells, as well as T cells and macrophages. It is not clear, since T cells need restimulation in vitro and in vivo, whether T-B restriction reflects T-macrophage restrictions. Thus, F1 helper cells induced in vivo or in vitro with macrophages of one parental strain are restricted to interacting with anti-theta treated (T cell-depleted) spleen cells of the same, but not the other, parental strain of the F1. (Text Abridged)

378. THE ROLE OF MACROPHAGES IN ALLOGRAFT IMMUNITY

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The present application will pursue the following objectives: (1) Prolongation of allograft survival after in vitro culture: a) To determine the optimal (least toxic to other cells) tissue culture conditions which will kill donor leukocytes (macrophages); b) to determine if these conditions will allow survival of thyroid and pancreatic tissues in tissue culture as evidenced by histological examination; c) to determine if these conditions will allow survival of thyroid and pancreatic tissues in tissue cultures as evidenced by functional tissue; d) to determine if the successful 'optimal conditions' found in part 2 will permit successful allogeneic thyroid and pancreas transplantation, first in the mouse and later in a larger animal, the Yucatan miniature pig. (2) The induction of arginase in macrophages: a) Purification of mouse liver and peritoneal cell arginase and production of antisera to these proteins; b) determine the specific cell type in the peritoneal cell population producing the arginase and determine whether the increase in arginase represents increased synthesis, decreased secretion or an unmasking of an inactive precursor; c) determine the source of calmodulin in serum; d) determine if calmodulin levels are increased in tumor bearing animals and patients.

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379. LYMPHOCYTE BINDING TO MACROPHAGES

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The binding of murine B and T lymphocytes to macrophages will be studied. The binding of a subpopulation of B cells will be studied in detail and the functional correlates of their binding determined. The subpopulation of T cells that regulates B cell binding will be identified.

The T cell population that can bind to macrophages under antigen-free conditions will be identified. T cell that can bind to allogeneic or antigen pulsed macrophages will be phenotyped.

The roles of lymphocyte-macrophage binding in lymphocyte differentiation will be studied.

BIBLIOGRAPHIC REFERENCES: Mongini, P., Friedman, S., Wortis, H.H. Accessory cell requirement for anti-IgM induced proliferation of B lymphocytes. Nature 276: 709, 1978.

380. MONOCLONAL ANTIBODY DEPLETION OF MACROPHAGES IN VIVO

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The goals of the proposed research are to: (1) prepare monoclonal anti-mononuclear phagocyte antibody that will effect and sustain the selective depletion of mononuclear phagocytes in vivo, and (2) determine the effects of such sustained depletion on the course of tumor regression or progression. Starting with hybridomas already on hand that produce rat monoclonal antibody against mouse mononuclear phagocytes, we will first select antibodies that detect antigens found only on cells (subpopulations) of the mononuclear phagocyte differentiation series. Of these antibodies, we will identify those that will selectively interfere with the mobilization of mononuclear phagocytes in the inflamed peritoneal cavity. Such an in vivo assay is necessary because the mechanism through which depletion will be effected is unknown, and it is likely that the majority of intratumoral macrophages arise through the influx and maturation of blood monocytes. A sustained in vivo effect will be established by reducing the immune responsiveness of recipient mice to rat Ig, thereby diminishing or eliminating the problems of immune elimination and immune complex disease. When we have developed the capability to sustain the selective depletion of mononuclear phagocytes in vivo, we will investigate the effect of doing so on the course of either tumor regression or progression. If depletion can be maintained for an appropriate period of time, these studies will include the effect of mononuclear phagocyte depletion on the rates of induction and progression of neoplasms that are caused by the application of various chemical carcinogens.

381. ANTIGEN PROCESSING BY MACROPHAGES

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This is part of a broader project. A summary of this subproject is not available.

D. OTHER STUDIES ON MONOCYTES AND MACROPHAGES

382. SURFACE MARKERS OF BENIGN AND MALIGNANT MONOCYTES

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Springer et al. have recently purified a rat spleen cell-mouse myeloma hybrid clone that secretes a monoclonal IgG antibody (M1/70) which has as its antigenic target a discrete molecular species that has been found to be present as a major component of the mouse macrophage cell surface, but is absent on mouse lymphocytes. We have found that this antibody (designated antimac-1) cross-reacts with human monocytes and granulocytes, and hence provides a specific cell surface marker for further classifying subtypes of myeloproliferative diseases, and for identifying the cell of origin in malignant diseases for which this information is currently not known. We propose to study the distribution of the mac-1 antigen on cells isolated from normal human peripheral blood, bone marrow, and spleen using the techniques of immunofluorescence and the fluorescent-activated cell sorter. We plan on investigating the presence of this antigen on the neoplastic cells of patients with acute non-lymphocytic leukemia and the myeloproliferative diseases, as well as on the malignant cells isolated from the lymph nodes of patients with lymphoma.

383. CHARACTERIZATION OF HUMAN BLOOD MONOCYTE AND MACROPHAGE CYTOCIDAL POTENTIAL

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The physiology of human monocyte, macrophage and lymphocyte is under investigation in several major areas: 1) Human monocyte-macrophage capacity to kill and inhibit tumor cell proliferation continues under investigation. New findings in this area include: a) Pulmonary macrophages are actively cytocidal and cytotoxic for tumor cells and tumor cells from smokers are more active than those from non-smokers. b) Human macrophages which readily kill tumor cells do not kill normal, contact inhibited cell strains. 2) Investigation of the role of monocytes in modulating lymphocyte proliferation has led to several new findings: a) monocytes and macrophages inhibit the induction of suppressor T-lymphocytes in human mixed lymphocyte cultures (MLC), and b) a major monocyte product which enhances lymphocyte proliferation in MLC is lysozyme.

384. MEMBRANE FLOW AND THE MOUSE MACROPHAGE FC RECEPTOR

Mellman, I. S., Rockefeller University, Cellular Physiology & Immunology Lab., *66th & York Ave., New York, New York,* 10021, United States of America

The overall objective of this project is to perform a detailed analysis of macrophage plasma membrane (PM) function - biogenesis, turnover, and flow during endocytosis. However, these studies will concentrate on the Fc-receptor, an immunologically important PM protein, as a model membrane component. This receptor binds the Fc domain of certain IgG molecules and is known to function in the endocytosis of immune complexes and opsonized particles as well as in the antibody dependent cytolysis of tumor cells. Using a series of recently isolated rat monoclonal antibodies directed against the mouse (trypsin-resistant) Fc receptor and other undefined macrophage PM proteins, I will examine their synthesis, degradation, and internalization in radiolabelled cells. Of interest will be a determination of whether PM proteins internalized during endocytosis are degraded or recycled back to the cell surface. I have previously developed methods for the specific labelling (by iodination) of endocytosed PM and for the quantitative detection (by immune precipitation) of cell surface vs. intracellular antigen molecules which will be of great use in performing these studies. I also plan to purify the Fc receptor from a mouse macrophage cell line (J774) using the monoclonal anti-receptor antibody as an affinity reagent. Pilot studies using radiolabelled cells have indicated the feasibility of this approach: the receptor molecule apparently being a 60,000 dalton glycoprotein. Its in vitro translation will be attempted using J774 mRNA. These studies will provide the first direct molecular information regarding the nature of the macrophage Fc receptor.

385. ISOLATION OF MACROPHAGE AGGLUTINATION FACTOR

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Many human and animal diseases, including cancer, are associated with changes in cellular immunity (delayed hypersensitivity) and in production of lymphokines (non-antibody mediators of cellular immunity). It has been difficult in the past to quantitate these changes accurately in vitro, a difficulty which might be overcome if there were an antibody available which was specific for a lymphokine associated with cellular immunity; this antibody could be used to develop a radioimmunoassay to measure cellular immunity in vitro to tumor and other antigens. Macrophage agglutination factor (MAggF) is a lumphokine derived from antigen stimulated guinea pig lymph node cells with cellular immunity to antigen; similar activities have been reported in appropriately stimulated culture supernates of human and animal mononuclear cells. The chemical and physical nature of this lymphokine are poorly understood and its exact role in cellular immune reactions in vivo remains undefined. The proposed project will attempt to isolate guinea pig MAggF from concentrated culture supernates of antigen-stimulated guinea pig lymph node cells by sequential fractionation of these supernates by affinity chromatography over insolubilized gelatin, preparative isoelectric focusing and gel filtration chromatography. Isolated and purified active MAggF will be characterized and used to raise antisera in rabbits. The specificity of these antisera for MAggF will be determined. The characterized antisera will be then used to evaluate the role of MAggF in vivo cellular immunity and to quantitate in vitro models of these reactions.

386. NATURE OF T CELL AND MACROPHAGE FACTORS (MEDIATORS OF CELL INTERACTION)

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The summary for this project is under entry no. 148.

387. PHORBOL MYRISTATE ACETATE AS A PROBE FOR MACROPHAGE FUNCTION IN INFLAMMATION

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Lymphocyte activating factor (LAF), produced by activated macrophages, can replace the macrophage requirement for many T lymphocyte functions and its production is therefore of probable importance for amplification of many cell mediated immune responses. A study of the cellular mechanism of LAF production is being undertaken in an effort to better understand this amplification mechanism and how it is regulated. The mechanism of stimulation of LAF production by the tumor promoter and inflammatory agent, phorbol myristate acetate (PMA), will be examined as an approach to the general cellular mechanism by which more complex macrophage activators work.

Since the cellular mechanism of action of PMA has not been clearly established in any system, a cultured line of macrophages, P388D1, will be incubated with 3H-labeled PMA in an attempt, first, to identify the PMA receptor mediating LAF production. These studies will include demonstration that the PMA binding is specific and saturable at concentrations consistent with those giving rise to LAF production, correlation of binding affinity of structural analogs of PMA with LAF stimulating activity, and localization of the cellular binding component. A second series of experiments, studying effects of the binding on cellular functions leading to LAF production, will include examining effects of RNA and protein synthesis inhibitors. Depending on these results, further studies may include examination of cyclic nucleotide levels and various enzyme activities. Lines of P388D1 cells resistant to PMA induced LAF elaboration will also be generated and studied to help define the mechanism of LAF production. Effects of glucocorticoids, which inhibit elaboration of some macrophage products, and of retinoic acid, which blocks many PMA effects, will be examined to help characterize regulation of the system.

388. MACROPHAGE AND TUMOR CELLS - PATHOLOGIC STUDY

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Mononuclear phagocytes have important functions in the host's defenses against neoplasia. When mononuclear phagocytes have been activated, they can selectively destroy neoplastic cells in vitro. We have recently found that macrophages secrete a cytolytic protease which preferentially lyses neoplastic as opposed to non-neoplastic cells. We have furthermore found that activated, as opposed to non-activated, macrophages selectively bind neoplastic cells. Evidence of two sorts indicates that the ability to secrete this protease and the ability to bind the tumor cells are both necessary for macrophage-mediated lysis to occur. We are currently investigating the hypothesis that macrophage-mediated lysis is a multi-step event, encompassing first binding of the tumor cells to the macrophages and second secretion of lytic effector molecules by the macrophages.

BIBLIOGRAPHIC REFERENCES: Adams, D.O.,: Effector mechanisms of cytolytically activated macrophages. I. Secretion of neutral proteases and effect of protease inhibitors. J. Immunol. 124:286, 1980. Adams, D.O., et al. Effector mechanisms of cytolytically activated macrophages. II. Secretion of a tumor-selective cytolytic factor by activated macrophage and its relationship to secreted neutral proteases. J. Immunol. 124:293, 1980

389. TUMOR DEFENSE BY PLATELETS AND MACROPHAGES

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Our goal is to study the mechanisms of immune defense against tumors. More specifically we plan to carry out the following projects: (a) Studies on the mechanisms of macrophage activation, especially the role of prostaglandins in this reaction. (b) Isolation and purification of macrophage activating factor(s) from stimulated lymphocytes. (c) Further studies on the mechanisms of antigen recognition by T lymphocytes. (d) Studies on the mechanisms of action of lymphocyte activating factor. (e) Isolation and characterization of human leukemia specific antigens. (f) Purification and physicochemical characterization of a macrophage-membrane associated cytotoxic factor.

BIBLIOGRAPHIC REFERENCES: Pasternack, G., Johnson, R., and Shin, H.S., Tumor cell cytostasis by macrophages and antibody in vitro: I. Resolution into contact dependent and contact independent steps. J. Immunol., 120, 1560, 1978. Johnson, R., Siliciano, R. and Shin, H., Suppression of antibody-sensitized tumor cells by macrophages: Insufficient supply or activation of macrophages within large tumors. J. Immunol., 122, 379, 1979.

390. ANTITUMOR ACTION OF PHAGOCYTES AND LYMPHOCYTES

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The ability of macrophages to release hydrogen peroxide is probably the first correlate of macrophage activation which has been shown to be both: (a) increased in macrophages with enhanced antitumor and antimicrobial activity; (b) low in macrophages with low levels of antitumor and antimicrobial activity; (c) in itself capable of antitumor and antimicrobial activity, which in some cases is sufficient to account for the activity of the macrophages; and (d) defined and quantifiable at a biochemical level.

The objective of the work proposed here is to extend these findings. Such studies will seek to learn whether antibody-dependent cell-mediated cytoxicity by macrophages, and T cell-mediated cytotoxicity have an oxidative basis; how tumor cells resist different oxidative species; whether peroxide-resistant variants can be selected; whether tumors suppress macrophage oxidative responses; whether H2O2 can kill tumor cells in vivo; whether certain new agents trigger H2O2 release from activated macrophages; and whether the oxidative response of leukocytes may activate certain cancer chemotherapeutic drugs. Human studies will focus on improved methodology for collection of blood monocytes and emigrated mononuclear phagocytes from skin windows, comparing their oxidative and antitumor responses before and after activation by lymphocyte mediators. Biochemical studies will attempt to localize and partially characterize the oxidase, and explore the prolonged kinetics of the oxidative response, in macrophages.

BIBLIOGRAPHIC REFERENCES: Nathan, C.F., Brukner, L.H., Silverstein, S.C. and Cohn, Z.A. Extracellular cytolysis by activated macrophages and granulocytes. I. Pharmacologic triggering of effector cells and the release of hydrogen peroxide. J. Exp. Med. 149: 84-99, 1979. Nathan, C.F., Silverstein, S.C., Brukner, L.H., and Cohn, Z.A. Extracellular cytolysis by activated macrophages and granulocytes. II. Hydrogen peroxide as a mediator of cytotoxicity. J. Exp. Med. 149: 100-113, 1979.

391. LYMPHOCYTES AND MACROPHAGES IN NEOPLASTIC DISEASES OF THE LYMPHOID SYSTEM

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The summary for this project is under entry no. 182.

392. STUDIES ON THE DIFFERENTIATION AND FUNCTIONS OF MACROPHAGES

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The aim of the proposed project is to further understand the role of macrophages in immune responses in normal and in tumour bearing animals. In recent years it has become apparent that macrophages are functionally diverse cells which are necessary participants in many different immune mechanisms. Evidence has accumulated supporting the hypothesis that functionally distinct subpopulations of macrophages exist. Analysis of the role of macrophages in immunity has been limited by lack of knowledge of the differentiation of macrophages, inability to precisely define the cells and problems in manipulating macrophages by conventional means. The subpopulation hypothesis will be tested by preparing monoclonal antibodies to macrophages 'activated' for tumor cytolysis. The antigens monospecifically defined in this manner will be studied biochemically, for tissue distribution, and as markers for separating tumoricidal and antigen presenting subpopulations in the fluorescence activated cell sorter. The antibodies will also be studied for their ability to block macrophage functional activity.

393. MACROPHAGE IDENTIFICATION AND PROGNOSTIC SIGNIFICANCE IN HUMAN MAMMARY AND COLONIC CANCERS

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The purpose of this project is threefold: 1. To develop monoclonal antibodies to human macrophages. 2. To use those antibodies in quantifying the number of macrophages and their distribution in human mammary and colonic cancers. 3. To determine whether there is any correlation between macrophage content of these tumors and prognosis.

III. MULTIPLE AND OTHER CELL TYPES INVOLVED IN THE TUMOR IMMUNE RESPONSE

394. ROLE OF LYMPHOCYTES AND MACROPHAGES IN HOST-TUMOR RELATIONSHIP

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OBJECTIVE: Lymphocyte subpopulations, monocytes, and tissue macrophages were studied in order to evaluate their quantitative and/or functional involvement in some groups of tumor patients.

APPROACH: A) Isolation and identification of macrophages from normal and tumor tissues; b) monocyte isolation by adherence; c) identification and purification of lymphocyte subpopulations; d) functional exploration (chemotaxis, natural cytotoxicity) of purified cell populations.

PROGRESS: 1) The number of tumor-infiltrating macrophages was estimated in patients with squamous and basal cell carcinomas. The macrophage content of basal cell tumors was significantly higher than that found in squamous cell carcinomas. The degree of macrophage infiltration was also established in a group of 45 patients with bladder carcinoma. A lower macrophage content seemed to be consistent with a poorer prognosis and a higher frequency of recurrences and/or metastases. 2) Using monocytes and purified lymphocyte subpopulations as effector cells, natural cytotoxicity was found to be higher in non-T and low avidity T-lymphocyte fractions obtained from patients with bladder carcinoma, and such activity could be increased by BCG immunotherapy. 3) T-lymphocytes from a group of patients with B-cell CLL showed decreased response to ConA and PHA; response was restored after addition of autologous monocytes. Moreover, CLL T-lymphocytes failed to help differentiation of normal, PWM-stimulated B-lymphocytes into plasma cells, even when normal monocytes were added to culture. 4) Monocyte and macrophage chemotaxis was performed using as chemotactic agents zymosan-activated fresh serum and a factor obtained by stimulation of cultured leukocytes with ConA. Monocytes from patients with various tumors showed a marked decrease in chemotactic activity, which was almost completely restored after surgical resection of the tumor. Macrophages isolated from tumor fragments showed a defective chemotaxis, which was further inhibited by soluble factor(s) obtained from sonicated tumor tissues. Moreover, such factor(s) inhibited chemotaxis of normal macrophages, and monocytes from normal and tumor patients.

395. NEW METHOD FOR SEPARATING LEUKOCYTE SUBPOPULATIONS

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We shall develop a method for separating human peripheral blood mononuclear leukocytes into different subpopulations by methods which will recover cells in high yield and purity and which will not impair their functional integrity. Reagents which have high affinities for specific cell surface components will be covalently bound to iron containing microspheres. These will be mixed with heterogeneous populations of leukocytes and the mixtures passed through a magnetic field. The cells which have surface receptors for the reagents attached to the microspheres will be retarded and separated from the remainder of the cells. Sequential separations will yield B lymphocytes (and B lymphocyte subpopulations), T lymphocytes (Fc rec positive and Fc rec negative) and monocytes. Cells obtained in this manner will be characterized with respect to surface markers and functional integrity. The acquisition of these purified cell populations will allow studies of the roles of specific cell types in immune functions, specific cell interactions essential to certain immune functions, and assessment of the numbers and functional integrity of specific cell types important to immune function in patients with neoplastic diseases.

396. IDENTIFICATION OF HUMAN AND ANIMAL LYMPHOCYTE SUBPOPULATIONS

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There is currently a substantial body of evidence in favor of functionally heterogeneous subpopulations of T, B, and Null lymphocytes. Recent work by L. Chess and S. F. Schlossman has shown that these subpopulations can be delineated by highly specific antisera and isolated by combinations of extremely sensitive techniques such as cellular immunoabsorbent chromatography, rosette depletion and the fluorescence activated cell Sorter. These techniques will be employed to further characterize the Null cell subpopulations of human and animal lymphocytes with regard to surface determinants, antibody and lymphokine production, cellular immunity, memory and suppression, tumor cell killing, and recirculation patterns. It is hoped that the differentiation pathways and heterogeneity of thymocytes, peripheral T cells, and antibody-producing cells will be further clarified since Null lymphocytes may act as precursors for several or all of these lymphocyte subpopulations and may share functions and determinants with some leukemic and lymphoma lines.

397. LYMPHOCYTE AND MONOCYTE MARKERS IN HUMAN MALIGNANT TISSUES

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OBJECTIVE: To detect and characterize markers for T- and B-lymphocytes, monocytes and tumor cells in human malignant tissues.

APPROACH: Haemadsorption to tissue sections and rosette assays with single cell suspensions are used to test for receptors for immunoglobulins, complement and erythrocytes. Indicator cells consist of sheep erythrocytes (E) as markers for T-lymphocytes, E sensitized with IgG antibody (EA) as markers for Fc receptors, and E sensitized with IgM antibody and human complement (EAC) as markers for complement receptors. Tissue sections, single cells in suspensions and short-term culture cells are treated in various ways and by various agents in order to distinguish between the receptors in malignant tissue and normal lymphoid tissue. Suitable techniques are elaborated, and their usefulness compared with that of established techniques.

PROGRESS: The elaborated technique with tissue sections is very useful for study of membrane markers in normal lymphoid tissue, and host cells infiltration in malignant tissue can easily be investigated. All malignant tissues express strong Fc receptor activity. This activity is very similar to the corresponding activity in normal lymphoid tissue. However, the 'malignant' receptor differs in some properties, strongly indicating the presence of unique receptors on the malignant cells for IgG. Apparently, there is a very minimal infiltration of normal host cells in malignant tissues. Studies on the relationship between the Fc receptor activity in malignant tissues and the clinical course are in progress.

BIBLIOGRAPHIC REFERENCES: Matre, R., Tonder, O., Thunold, S. and Solhaug, J.H; Properties of Fc receptors in normal and malignant human tissues. Scand. J. Immunol. 5, 361-368, 1976. Matre, R.: Specificity of Fc gamma receptors in human malignant tissue and normal lymphoreticular tissue. Scand. J. Immunol. 5, 1976.

398. STUDIES OF LEUKOCYTE AND PLATELET MEMBRANE RECEPTORS

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The project will focus on the structure and function of leukocyte membrane receptors and surface antigens. The project has four interrelated long-term objectives. The first of these is to characterize normal leukocyte membrane surface markers for their distribution on different leukocyte types and for their order of appearance during leukocyte maturation. The project will not be restricted to one leukocyte type, but will encompass the spectrum of bone marrow nucleated cells, including erythroid precursors and megakaryocytes. The findings on normal leukocytes form the basis for the second objective of the project, to find and characterize leukemia specific membrane markers and determine which of these may have prognostic significance for early determination of important clinical subtypes or stages of leukemic disease. The third goal is to analyze lymphocyte membranes by scanning electron microscopy and determine the distribution and association of different types of receptors on individual cells. The final goal of the project is to study platelet membrane receptors for IgG, Fc (Fc receptors) and determine the relationship of these receptors to platelet receptors for clotting factor VIII. Preliminary studies have suggested that the Fc receptor may be either the same entity as the factor VIII receptor or located adjacent to it. Since IgG containing immune complexes have been shown to block factor VIII receptor activity, platelets from patients with immune complex-associated disease will be examined for platelet dysfunctions caused by a common blockade of Fc and factor VIII receptors.

399. FACTORS INFLUENCING THE FUNCTION OF TRANSFUSED GRANULOCYTES

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Preliminary evidence has been developed which demonstrates that serologic factors exist in neutropenic infected leukemic patients which: a) Alter chemotaxis of donor granulocytes; b) reduce phagocytosis of oil droplets incubated in recipient serum; and c) predict, to some extent, outcome (fatal or nonfatal) of anti-infective therapy. The proposed study has as its objective to enlarge upon those observations, to test whether: a) Serologic factors predict clinical events related to the transfusion of granulocytes, and b) in vitro studies reflect in vivo behavior of transfused granulocytes.

Methods will consist of: a) Determining the effects of recipient serum on donor granulocytes in chemotaxis and oil-red-O opsonization assay systems; b) labelling the donor granulocytes with Indium 111 and observing in the recipient: 1. Ability of labelled cells to migrate into skin chambers. 2. Intravascular survival and kinetics of labelled donor cells. 3. Final distribution of label with gamma imaging.

Finally, clinical parameters will be followed and entered into a computer system for later correlative analysis. In addition to standard parameters, forced sorting methods for quantifying clinical observations will be collected and cumulative sum-plotting of the temperature curve will be used.

Logistic regression and Cox modeling approaches will be used to deal with the data generated.

The ability to identify in advance those patients who will do poorly with broad spectrum antibiotic therapy and granulocyte transfusions will permit a concentrated effort to be directed at those factors which are responsible for this in the hopes of improving survival in neutropenic infected patients.

400. DEVELOPMENT OF GRANULOCYTE BANKING PROCEDURES

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Proposed research concerning the development of practical procedures for short-term and long-term banking of granulocytes effective for hematologic support of leucopenic patients is a continuation of our long-range program. Using our centrifugal elutriation method for isolation of pure granulocytes, we will test the following new approaches to improve granulocyte preservation such as: (1) Maintaining cells in a resting metabolic state by lactate inhibition of phosphofructokinase. (2) Maintaining intracellular levels of nucleotides (ATP and cyclic AMP). Restoration of nucleotides intracellularly by means of liposomes or glycerol will be studied. (3) Macromolecular plasma substitutes will be used in place of serum proteins in preservation medium. (4) Reduction of granulocyte damage due to intracellular release of lysosomal enzymes by lysosomal membrane stabilizers and/or enzyme inhibitors. (5) Regeneration of -SH groups of cells by sulfhydryl compounds after preservation. (6) Modification of granulocyte membrane lipids by increasing proportion of saturated lipids using liposomes. Also, we will investigate effects of various gels for stabilizing cell suspension during storage. Furthermore, we will examine several recent claims of successful recovery of human granulocytes after cryopreservation and will adopt any repeatable effective procedure as a base for improvement. Animal model, using rabbits, will be developed for testing in vivo granulocyte function.

401. DIFFERENTIATION OF GRANULOCYTES AND MONOCYTES

Metcalf, D., Burgess, A. W., Watt, S. M., Nicola, N. A., Cooper, P., Walter & Eliza Hall Inst. for Medical Research, *c/o Royal Melbourne Hospital, Melbourne, Victoria 3050, Australia*

The summary for this project is under entry no. 369.

402. KINETICS AND CONTROL OF HEMATOPOIETIC STEM CELLS AND NEUTROPHILIC GRANULOCYTES

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The proposed project is to continue the study of factors controlling the proliferation and maturation of hematopoietic stem cells. Particular emphasis is placed on the study of the in vitro proliferation of granulocytic progenitor cells (CFU-C) from the blood and marrow of animals and man. Colonies of granulocytes and mononuclear cells are cultured from marrow cells in a soft gel culture system in the presence of a colony stimulating activity (CSA).

Previously we and other laboratories demonstrated that the monocyte-macrophage system is a major source of CSA. More recently lymphocytes stimulated with mitogens or specific antigens have been shown to release CSA. A specific eosinophil stimulating CSA has been obtained from sensitized lymphocytes. Other studies have indicated that lithium carbonate induces leukocytosis which is probably mediated through increasing CSA release from monocytes. Studies are being continued to 1) characterize the properties of eosinophil CSA, 2) determine the in vivo effect of eosinophil CSA, 3) determine kinetic changes of neutrophils during lithium-induced neutrophilia.

Other studies concerned with granulocytic progenitor cells include 1) determination of the role of suppressor lymphocytes in aplastic anemia and neutropenia; 2) quantitation of stem cells in aplastic anemia and neutropenia; 3) the study of the relationship of colony formation in polycythemia vera, myelofibrosis, and leukemia; and 4) the correlation of in vitro colony formation with response to therapy and course of survival in leukemia.

403. PHYSICAL CHEMISTRY OF LYMPHOCYTE ACTIVATION

DeLisi, C., Siraganian, R., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Mathematical Biology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

A biological theory of the kinetics of activation and specific desensitization of basophils from allergic and immunized individuals has been developed, and applied to the analysis of a wide variety of data.

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404. AN INVESTIGATION OF THE FUNCTIONS OF MAST CELLS IN LYMPH NODES, WITH SPECIAL REGARD TO THE LYMPHATIC SPREAD OF LYMPHOMA CELLS

Stoddart, R. W., Anderson, J. C., University of Manchester, School of Medicine & Dentistry, Dept. of Experimental Pathology, *Bridgeford St., Manchester M13 9PL, England, United Kingdom*

OBJECTIVE: The purposes of this study are: 1) to achieve selective labelling of the products of a metabolically distinct population of mast cells found in lymph nodes; 2) to identify the nature of the glycosylated macromolecules they produce; 3) to explain the possible effects of mast cell products on lymphocytes and other lymph node cells; and 4) to seek abnormalities that malignant lymphocytes and lymphoblasts may show in their responses to this substance.

APPROACH: Use will be made of tissue slice techniques, radioactive labelling with sugars and sulfate, metabolic manipulation, and autoradiography (optical and EM) to obtain selective labelling of mast cell products. These products will be separated and analyzed by a range of electrophoretic and electrofocusing procedures, and enzymatic and chemical methods. By using in vitro and in vivo methods, lymphocyte recirculation and segregation, and high endothelial cell function will be studied in relation to mast cell activity. Both human and rat leukemias and lyphomata will be used to relate these observations to the patterns of spread of lymphoid neoplasms.

PROGRESS: This is a new project.

405. BASOPHIL AND MAST CELL FUNCTION IN THE CONTROL OF CANCER

Dvorak, A. M., Dvorak, H. F., Galli, S. J., Gang, D. L., Massachusetts General Hospital, Dept. of Pathology, *32 Fruit St., Boston, Massachusetts*, 02114, United States of America

The purpose of this investigation is to elucidate the function of basophils and mast cells in cell-mediated hypersensitivity reactions, particularly with regard to an understanding of the role of these cells in immunologic tumor rejection. The experiments proposed will make use of a tissue culture system and will employ a combined morphologic, immunologic, and biochemical approach to determine: 1) Whether sensitized lymphocytes or their products affect pharmacologic mediator (histamine) synthesis and/or release in basophils/mast cells, or, conversely, whether basophils modulate lymphocyte function; 2) the mechanism(s) of mediator release from basophils in both physiological and pathological circumstances, and as mediated by cellular or humoral pathways; 3) the fate of basophils in tissue culture following anaphylactic degranulation; 4) the effect of basophils and their pharmacologic mediators on tumor cells; 5) the effects of basophil ablation, induced by a specific antiserum, on the expression of cellular immunity.

406. SYNTHESIS AND RELEASE OF POLYPEPTIDE HORMONES BY MAST CELLS

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Mast cells are ubiquitously distributed throughout mammalian organs in association with connective tissue. Special consideration is being given to the role of mast cells in the lung where these cells are present beneath the epithelium in trachea and bronchi as well as in mucous lining. Mast cells contain bioactive amines such as histamine and serotonin, sulfonated glycosaminoglycans, e.g., heparin, and peptide chemotactic factors, as well as polypeptide hormones such as vasoactive intestinal polypeptide, corticotropin (ACTH) and beta-lipotropin (beta-LPH). This investigation then focuses on: (1) the characterization of the precursor of mast cell ACTH and its comparison to that from pituitary glands; (2) the in vitro synthesis of peptide hormones in mast cells; (3) the processing and release of ACTH and beta-LPH by bioactive agents and peptide hormones; and (4) the use of a mastocytoma cell line to study the effect of reversible granulopoiesis on the synthesis, storage and release of an enkephalin precursor. Studies using a mouse mastocytoma have shown that this tumor contains numerous polypeptide hormones. Therefore, it appears to have the capacity for ectopic synthesis of peptide hormones analogous to that observed in various pulmonary tumors.

407. CHARACTERIZATION AND FUNCTIONAL STUDIES OF 'A' CELLS

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The roles of 'A' cells as effector cells in tumor cell growth regulation and as accessory cells in antibody synthesis are to be studied in light of their presence in the light density PE subpopulation that is enriched for both these activities. 'A' cells will be characterized as either possible precursor cells for macrophages or as a unique cell type. The studies will include the examination of the 'A' cells for surface markers common to macrophages and lymphocytes as well as for antigenic and functional markers, and for their ability to differentiate into macrophages in culture. In addition, bone-marrow derived macrophage colonies will be examined for the presence or absence of 'A' cells in an effort to determine whether macrophages and 'A' cells share a common stem cell.

408. NEUTROPHIL FUNCTION

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This is part of a broader project. A summary of this subproject is not available.

409. DEFECTS IN IMMUNOREGULATORY CELL INTERACTIONS IN PATIENTS WITH IMMUNE DYSFUNCTION

Waldmann, T. A., Broder, S., Fleisher, T. A., Misiti, J., Greene, W., Uchiyama, T., Korsmeyer, S., Leder, P., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Metabolism Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Studies were directed toward determining the steps involved in the differentiation of B cells into immunoglobulin synthesizing and secreting plasma cells. Special emphasis was laid on developing new techniques to study the role of helper T cells, macrophages, and suppressor cells in these immune regulatory processes and to define defects in these immunoregulatory cell interactions in patients with immune dysfunctions. Recombinant DNA technology has been applied to study the arrangement and rearrangement of immunoglobulin genes in lymphocytic leukemias and lymphocyte cell lines. Leukemias of both suppressor and helper T cells have been identified. Excessive numbers of suppressor T cells have been demonstrated in association with agammaglobulinemia, selective IgA deficiency, suppressor leukemias, infectious mononucleosis, and post-transplantation immunodeficiency state. In studies of leukemias and cell lines, it was shown that human B-cells rearrange their expressed allele and may retain, rearrange, or delete the non-expressed allele. Unexpectedly the kappa gene was deleted in lambda expressing B cells.

BIBLIOGRAPHIC REFERENCES: Waldmann, T.A., Broder, S., Blaese, R.M., Durm, M., Goldman, C., and Muul, L.: The role of suppressor cells in human disease. In Dosh, H., and Gelfand, E. (Eds.): The Biological Basis for Immunodeficiency. 1980, pp. 223-239. Broder, S., Mann, D.L., and Waldmann, T.A.: Participation of suppressor T cells in the immunosuppressive activity of a heteroantiserum to human Ia-like (P12, 30) antigens. J. Exp. Med. 151: 257-262, 1980.

410. INDUCTION OF DIFFERENTIATION IN HUMAN LEUKEMIC CELL LINES

Verbi, W., Robinson, J., Boss, M., Asser, U., Greaves, M. F., Imperial Cancer Research Fund, Membrane Immunology Laboratory, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

We have attempted to reverse maturation arrest in established lines of human lymphoid and myeloid leukemic cells using ligands known to be effective in animal leukemias, e.g., DMSO, butyric acid, vitamin A in Friend cells or rat myeloid cells. As previously published, HL-60, a promyelocytic line, undergoes terminal differentiation to granulocytes with DMSO. This change is accompanied by quantitative alterations in membrane antigen expression (HLA-A,B; 'myeloid' antigen, C3 receptor) as well as ultrastructural and enzymatic (maturation). K562, a presumed 'myeloid' cell, was found to have cell surface glycophorin, a red cell membrane marker, and undergoes at least limited erythroid differentiation with DMSO or butyric acid. ML-1, a line established from AML, can be induced to undergo at least partial (-promyelocytic) maturation with DMSO. Surprisingly, the resting culture was found to include micron chain (IgM) containing HLA-DR plus (DA2) blast cells which we presume to be pre-B cells. If this is confirmed by biosynthetic evidence of Ig synthesis and if current karyotypic investigations confirm clonal identity, then this line must be maintained or initiated in a pluripotential stem cell as in Ph1 chromosome-positive leukemia (ICRF Scientific Report 1978 - report 224). Two non-T, non-B ALL lines (Reh, KM3) and a PH1 positive pre-B line (Nalm-1) have been extensively investigated for lymphoid maturation inducibility and to date no phenotypic changes have been observed.

411. BIOCHEMICAL MECHANISMS OF LEUKOCYTE CYTOTOXICITY

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This study will examine the biochemical mechanisms whereby three populations of human Fc receptor leukocytes (granulocytes, monocytes and K cells) carry out cytolysis to a variety of target cells including bacteria, fungi, and normal and malignant target cells. The metabolic characteristics of these effector cells in ADCC and NK assays will be determined as well as their ability to generate oxygen radicals (via stimulation with phorbol myristate acetate) which can kill bacteria, fungi and target cells. The molecular species responsible for target cell damage will be determined as well as characterization of membrane lipid changes which occur during oxidant injury. Finally, effector cells from patients with selective enzyme deficiencies in oxygen metabolism will be examined for ADCC and NK cytotoxic function.

412. GENETIC ANALYSIS OF NORMAL AND MALIGNANT LYMPHOCYTES

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This research has the objective of combining the present knowledge of immunogenetics, as applied to the major histocompatibility complex, with the technology of separation of different lymphoid cells to further characterize the HLA linkage group. These studies will help to identify alloantigen systems restricted to T lymphocytes, monocytes and B lymphocytes. We intend to study several families in which we have found HLA recombination or HLA-GLO recombinations and HLA-C2 or HLA-Bf recombinations by the HLA-D polymorphism in order to map the complement loci. In another group of experiments utilizing cellular reagents we will investigate functional aspects of T lymphocytes (helper and suppression) following identification of alloantigens on resting T lymphocytes or on activated T lymphocytes. We hope to divide the HLA-D region into different subregions which may code for help or for suppression of the immune system.

BIBLIOGRAPHIC REFERENCES: DeWolf, W.C., Lange, P.H., Einarson, M., and Yunis, E.J.: HLA and testicular cancer. Nature (Lond), Vol. 277, No. 5693:216-217, 1979. DeWolf, W.C., Carroll, P.G., Yunis, E.J.: The genetics of PLT response. I. Methodology for optimal PLT discrimination. J. Immunol. 122:860, 1979.

413. SENSITIZATION OF LYMPHOCYTES TO VIRUS TRANFORMED CELLS

Howe, M. L., State University of New York, Downstate Medical Center, School of Medicine, Dept. of Microbiology & Immunology, *450 Clarkson Ave., Brooklyn, New York*, 11203, United States of America

Recent investigations in our laboratory demonstrate that murine lymphoid cells have the capacity to recognize and respond to antigens of syngeneic tumor cells in an entirely in vitro culture system. Experiments will be performed to determine the role of T lymphocytes, B lymphocytes and macrophages in the initial recognition of tumor specific transplantation antigens (TSTA) in this in vitro system. We will also ascertain the role of these cell types in lysis of tumor cells following in vitro sensitization. In addition, studies will be conducted to determine the relationship between TSTA and normal cell surface antigens which are controlled by the major histocompatibility complex. It is anticipated that these studies will yield new information concerning the mechanisms of host defenses against neoplasia.

414. REGULATION OF LYMPHOCYTE ACTIVITY BY PROTEASES

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Several biological systems such as the complement cascade and the clotting systems are controlled by complex protease-protease inhibitor systems.

The objective of this research proposal is to investigate the role of such protease and protease inhibitor systems in the regulation of lymphocyte functions. This includes regulation of proliferation as well as differentiated functions. The approach to the problem will be multi-faceted. First, using short-term culture and various mitogenic stimuli, natural and synthesized protease inhibitors will be tested for their ability to interfere with proliferation of subpopulations of lymphoid cells. This aspect of the research will concentrate on alpha2-macroglobulin which we have shown to possibly be a regulatory molecule. Secondly, in addition to proliferation, the role of proteases and protease inhibitors in lymphocyte functions such as development of antibody secreting cells (primary and secondary cultures) and cytotoxicity will be investigated. Thirdly, the cell-surface modifications induced by proteolytic activation of lymphoid cells will be investigated biochemically using cell-surface iodination techniques. Fourthly, the biochemical nature of cell-surface proteases, which we and others have shown to occur on lymphoid cells, will be determined. The cell surface proteases on subpopulations of lymphoid cells will be characterized for substrate specificity, activity during lymphocyte activation, molecular weight and antigenic nature.

Results obtained from the proposed research will provide information on internal immunological regulation (T-cell-B-cell, B-cell-macrophage, etc.) as well as on the nature of possible immunological modifications induced by interaction with the environment under homeostatic conditions or in pathological conditions such as autoimmune disease states (arthritis) or cancer.

BIBLIOGRAPHIC REFERENCES: David A. Hart. Augmentation of Zinc Ion Stimulation of Lymphoid Cells by Calcium and Lithium. Exp. Cell. Res. 121:419 (1979).

415. REGULATION OF CYTOTOXIC ACTIVITY AGAINST TUMOR CELLS

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The goal of the proposed project is to determine if three classes of suppressor cells (i.e., macrophage-like, null-type, T lymphocytes) capable of inhibiting in vitro natural and induced cell-mediated cytotoxicity against tumor targets regulate these reactivities in vivo. These studies will include the autoreactive natural killer (NK) model against lymphoma and nontransformed target cells, and the autoreactive F1 hybrid anti-parent cell-mediated lympholysis model against lymphomas. Suppressor cells will be characterized as to origin and cell lineage, surface markers, responsiveness to physiologic or experimental stimuli, and mechanism of action. In vivo correlations will be sought so as to infer whether the suppressive mechanisms occur in vivo and result in a regulatory network for surveillance over leukemogenesis.

IV. ANTIBODIES

A. IDENTIFICATION AND CHARACTERIZATION OF ANTIBODIES

416. PATTERNS OF EXPRESSION OF LYMPHOCYTE SURFACE IMMUNOGLOBULINS IN CHRONIC LYMPHOCYTIC LEUKEMIA

Ling, N. R., Dhaliwal, H. S., University of Birmingham, Dept. of Immunology, *Birmingham B15 2TJ, England, United Kingdom*

OBJECTIVE: To attempt to correlate surface immunoglobulins in lymphoproliferative disease with other diagnostic features and with prognosis.

APPROACH: Testing of large numbers of CLL with a panel of surface marker tests, and particularly with a new and especially sensitive rosette test for SmIg. Gathering of general clinical information following course of disease.

PROGRESS: Large amount of data. Technically successful, but data yet to be analyzed. Some success in early diagnosis of B cell lymphoproliferative diseases.

417. LYMPHOMA IMMUNOGLOBULINS AS TUMOR SPECIFIC ANTIGENS

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In this project, we will be attempting to study cell surface antigens which are specific for tumors. For this purpose, we have adapted a technique for the production of antibodies of restricted heterogeneity in vitro. The objectives here will be to define the antigenic relationships between different closely related lymphoid tumors and to determine the degree of overlapping in the array of tumor specific antigens of these different tumors. In addition, we will be attempting to produce antibodies which recognize human tumor specific antigens, particularly in leukemia and lymphoma. These antibodies against human tumor antigens may find application in immunodiagnosis and immunotherapy.

418. CORRELATION OF MORPHOLOGICAL CHARACTERISTICS OF NON-HODGKIN'S LYMPHOMAS AND LEUKEMIAS WITH SURFACE IMMUNOGLOBULIN

Sukumaran, M. S., Majhi, U., Rajalakshmi, K. R., Cancer Inst., Dept. of Pathology, *Adyar, Madras 600020, Madras, India*

The study of cellular characteristics of tumors has acquired great significance for determining the prognosis of non-Hodgkin's lymphomas and acute lymphatic leukemias. There have been many attempts to classify these tumors, based on morphological, functional, or immunological characteristics, into T and B cell lymphomas and leukemias. None of these methods appears to be specific when used singly. The clinical behavior and the prognosis of the condition depends on the cell type. We intend to study the cellular characteristics by combining morphological, cytochemical and immunological methods, and to type these into B cell and T cell lymphomas or leukemias and correlate these findings with the clinical behavior.

The study of surface immunoglobulin is done by immunofluorescence technique using FITC labelled anti-IgG and IgM antisera. The tumor cell suspension is stained by direct method.

Impression smear from lymph nodes and peripheral blood smears are used for cytochemical study of PAS and acid phosphatase stain. Morphological study is done on imprints stained by Pap smear and also on histological sections. The study has just commenced.

419. ISOLATION, IDENTIFICATION AND PURIFICATION OF AN IGM DIFFERENTIATING PROTEIN IN LEUKEMIC CELLS FROM LEUKEMIC CELL LINES CULTURED IN VITRO

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OBJECTIVE: To isolate and purify a protein differentiator IgM from normal cells and myeloid leukemia cell lines with macrophagic characteristics, using induction by activation of cells with lipopolysaccharide products.

METHOD: The myeloid cells in continuous culture and having macrophagic characteristics are challenged with lipopolysaccharides of bacterial origin. Once the incubation supernate is obtained, the IgM protein is located using its capacity for forming colonies in agar from bone marrow cells. Using various chromatographic techniques the IgM differentiating protein is then purified.

PROGRESS: The optimal conditions for producing IgM have been identified; its purification is underway.

420. RICIN A-CHAIN/ANTI-CEA ANTIBODY CONJUGATES

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A novel conjugate consisting of Ricin A chain disulfide linked to the Fab' fragment of specific antibody has been constructed. The specific binding site on the antibody half of the molecule functions as a directional carrier, allowing A chain entry into the cytoplasm of cells bearing appropriate antigen, and expression of cytotoxic effects. Proposed is the construction of such a conjugate, utilizing affinity-isolated and hybridoma-derived anti-CEA (carcinoembryonic) antibody as the specific antibody. The specific cytotoxicity of such conjugates, constructed both with specific Fab' and intact IgG, will be evaluated in in vitro and in vivo tumor systems. The mechanism of intracytoplasmic entry of A chain following antibody surface binding will also be addressed.

421. CHEMICAL STRUCTURE OF IMMUNOGLOBULIN MOLECULES, ALLOTYPES AND HUMAN HISTOCOMPATIBILITY ANTIGENS (ABBREV)

Appella, E., Inman, J., Rogers, M., DuBois, G. C., Robinson, E. A., DeLeo, A., Jay, G., Ferrini, U., Seidman, J., Perham, R., Ramanathan, L., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cell Biology Laboratory, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

Chemical and Structural Studies of Antibodies, Histocompatibility Antigens, Tumor Antigens and Epoxide Hydrase: It is the purpose of this project to study the chemistry and structure of different molecules. The amino acid sequence of light and heavy chain immunoglobulins is obtained in order to gain some insight into the nature of antibody specificity and the generation of diversity. Biochemical isolation of histocompatibility antigens, tumor antigens and epoxide hydrase is carried out. The chemical structure of these proteins is being approached by micro methods and the relationship of the amino acid sequence to immunoglobulin structure or other known structures is being analyzed. Cloning of histocompatibility antigens is being approached in order to study the molecular organization of the system and permit a better understanding of the nature of the extensive polymorphism at the H-2 loci.

BIBLIOGRAPHIC REFERENCES: Law, L.W., Rogers, M.J., and Appella, E.: Tumor antigens on neoplasms induced by chemical carcinogens and by DNA- and RNA-containing viruses. Properties of the solubilized antigens. In Weinhouse, S., and Klein, G. (eds.): Advances in Cancer Research, New York, Academic Press, 1980, Vol. 32, pp. 201-235. Appella, E.: Amino acid sequence of the light chain variable region of M511, a phosphorylcholine-binding murine myeloma protein. Molecular Immunology, in press, 1980.

422. STUDIES ON THE STRUCTURE AND FUNCTION OF THE CONSTANT PORTIONS OF IMMUNOGLOBULINS

Segal, D. M., Jones, J. F., Dower, S. K., DeLisi, C., Siraganian, R., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Immunology Branch, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

(1) Detailed equilibrium and kinetic studies have suggested a mechanism by which multivalent immune complexes interact with Fc receptors. The mechanism emphasizes the role of receptor density in the affinity with which a cell binds multivalent ligands. (2) A fluorescent assay employing flow microfluorometry has been developed for quantitating Fc receptors on individual cells. The method is especially useful for studying heterogeneous populations of cells. (3) IgG Fc receptors were studied and compared with the IgE receptors on rat basophilic leukemia cells. IgG and IgE were bound by distinct receptors; the IgE receptor binds only IgE, while the IgG receptor binds both IgG and IgE. (4) P388D1 cells were found to internalize immune complexes rapidly at 37 degrees. Internalization also occurs for IgG-dimers, trimers, and perhaps monomers. Subsequent to internalization, IgG is degraded to a 50,000 M.W. component. (5) Effector and target cells have been studied in 'franked' ADCC assays. It has been found that mouse and rat spleen cells and human peripheral blood lymphocytes can serve as effectors in this system. Tumor cells from a human B-cell line can be lysed by franked rat and human cells.

BIBLIOGRAPHIC REFERENCES: Alexander, E. L., Titus, J. A. and Segal, D. M.: Human Leukocyte Fc (IgG) Receptors: Quantitation and Affinity Using Radiolabeled Affinity Cross-Linked Rabbit IgG. J. Immunol. 123: 295-302, 1979. Plotz, P. H., Kimberly, R. P., Guyer, R. L. and Segal, D. M.: Stable Model Immune Complexes Produced by Bivalent Affinity Labeling Haptens: In Vivo Survival. Molecular Immunol. 16: 721-729, 1979.

423. VARIABLE REGIONS OF BICLONAL IMMUNOGLOBULINS

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This study will analyze the monoclonal immunoglobulins in the serum of patients who have biclonal or triclonal gammopathy. We will ultimately determine if biclonal or triclonal immunoglobulins from the same patient share identical idiotypic determinants and whether the light or heavy chains of these proteins share identical VL or VH regions. The combinations of immunoglobulins studied to date include IgA(kappa) plus IgG(kappa); IgM(kappa) plus IgA(kappa) plus IgG(kappa); IgG1(kappa) plus IgG2(kappa) plus IgG2(kappa).

The N-terminal amino acid sequences up to residue No. 45 of the GR alpha- and gamma-chains are known. We will determine the remainder of the amino acid sequence of the VH regions by sequencing cyanogen bromide fragments of the two chains or tryptic peptides of cyanogen bromide fragments. The N-terminal amino acid sequence up to residue No. 40 of the two GR kappa-chains is known. We intend to determine the sequence of the remaining amino acids in the VL region of these two chains by sequencing peptides generated by digestion with trypsin.

With the triclonal proteins, we will determine if the three kappa-chains are identical by isoelectric focusing, mapping of iodinated tryptic and chymotryptic peptides and N-terminal amino acid sequence. We will determine the N-terminal amino acid sequence of the mu-, alpha-, and gamma-chains. If the sequences are identical, we will determine the sequence of the remaining amino acids in the VH regions of these chains from peptides produced by cyanogen bromide cleavage.

We have shown that the two IgG2 molecules in patient GT's serum possess identical idiotype determinants and that the IgG1 molecule possesses a related idiotypic determinant. We will analyze the heavy and light chains of these three molecules to determine if they have identical N-terminal amino acid sequences and ultimately, identical variable regions.

424. SEQUENTIAL EXPRESSION OF IMMUNOGLOBULIN IN B LYMPHOID DEVELOPMENT

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Differentiation of B lymphocytes is accompanied by the expression of the different classes of immunoglobulin in a partially ordered sequence. Descendants of individual cells appear to express the several classes of immunoglobulin in association with a single variable region, as detected by antiserum to the parental idiotype. Some studies suggest that surface IgD is a marker of early B lymphocytes, which can differentiate into cells producing any of the other classes of immunoglobulin, in association with the parental variable region. Whether surface IgD bearing cells, or surface IgD and IgM bearing cells, give rise to cells producing IgM, IgG, IgA, and IgD will be investigated in somatic cell hybrids resulting from the fusion of clonally derived human B lymphoid cell lines expressing only IgD, or IgD and IgM, with mouse myeloma cells. The resulting cell hybrids will be examined for production of human IgM, IgG, IgA, and IgD. Antisera to the idiotype of the immunoglobulin expressed by the human parental cell line will be used to determine whether all of the daughter hybrid cells produce the human parental variable region in association with the several classes of human immunoglobulin produced. Recent evidence suggests that B lymphoid tumors result from transformation of a clone of B cells, with malignant manifestations at all stages of differentiation. These studies of the sequential expression of immunoglobulin in B lymphoid development may provide a rational basis for examining the origin of B lymphoid malignancies.

425. GENETIC CONTROL OF ALLOTYPE EXPRESSION OF HUMAN IG

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The synthesis of immunoglobulin in vitro will be studied in human peripheral blood lymphocytes after short-term culture with pokeweed mitogen. Secreted Ig will be measured by isotope radioincorporation and immunoprecipitation. The effect of prednisolone treatment on Ig synthesis and secretion will be analyzed at different levels of pokeweed mitogen. Cell separation experiments and irradiation will be used to examine the contribution of each cell type. The relationship between steroid sensitive cells in normal peripheral blood and steroid-sensitive suppressor cells in iummnodeficiency will be studied.

BIBLIOGRAPHIC REFERENCES: Characteristics of Suppressor Cell Activity Appearing in Cocultures of Two Individuals with Immunodeficiency with Thymoma, by Litwin, S.D. in Scand. J. Immunol. 11:15, 1980.

426. ISOLATION AND CHARACTERIZATION OF ANTIBODIES TO COLLAGEN/PROCOLLAGEN

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OBJECTIVES: To produce high titer antibodies to the carboxyl-terminal propeptides of human type I procollagen. To produce high titer antibodies to the endopeptidase which specifically cleaves the carboxyl-terminal propeptides of human type I procollagen. To produce the above antibodies in sufficient quantities so that they may be made available to other investigators.

427. ISOLATION AND CHARACTERIZATION OF ANTIBODIES TO COLLAGEN, PROCOLLAGEN

Furthmayer, H., Yale University, School of Medicine, Dept. of Pathology, *333 Cedar St., New Haven, Connecticut,* 06510, United States of America

OBJECTIVE: Prepare and isolate immunochemically defined antibody reagents to collagen of types I, II, III, IV (basement membrane), AB (fetal collagen), and procollagen of types I and III which react specifically with human proteins. Prepare similar reagents specific for mouse collagen of types I, III, and IV (basement membrane).

428. STRUCTURE AND GENETICS OF ANTIBODY VARIABLE REGIONS

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The studies proposed in this application are directed toward defining the primary structural diversity among antibodies that are specific for the same antigen and determining the genetic mechanisms that generate the diversity. These issues will be addressed through electrophoretic, idiotypic, and amino acid sequence analyses of anti-streptococcal group A carbohydrate antibodies elicited in strain A mice at three levels of immunocompetence: (1) Early in ontologic development (neonates), (2) primary immunized (single antigenic stimulus) adults, and (3) secondary immunized repeated antigenic stimuli) adults. The substrates for these studies are hybridoma antibody products obtained by in vitro cell-fusion of immune spleen cells and antigen-simulated spleen fragments with plasmacytoma cells. These analyses will reveal the relative contribution to the functional antibody repertoire of germ-line encoded variability versus somatically acquired variability, and the respective roles of somatic development and antigenic stimulation in generating antibody diversity.

BIBLIOGRAPHIC REFERENCES: Ploegh, H.L., Cannon, L.E., and Strominger, J.L. (1979). Cell-free translation of the mRNAs for the heavy and light chains of HLA-A and HLA-B antigens. Proc. Natl. Acad. Sci. USA 76:2273-2277.

429. STRUCTURE AND CHEMICAL NATURE OF SPECIFIC ANTIBODY SITES

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The tertiary structures of antibody molecules are being investigated with the main purpose of elucidating the nature of the specific combining site of the antibody molecule and its relationship to the whole antibody molecule, to the subunit structure of the antibody, to the amino acid residues in the site, and to the amino acid sequences involved in determining specificity.

Correlations are being sought between the primary amino acid sequences and the tertiary structures of antibody molecules for different antibodies directed against the same haptenic group and for antibodies directed against various haptenic groups. Efforts are concentrated on that part of the sequence involved in the binding sites. To achieve this goal homogeneous antibody preparations are being studied rather than the heterogeneous preparations which have been studied in the past.

Studies are being done by subjecting homogeneous antibodies to controlled chemical alterations so that reactions taking place in the binding region can be distinguished from reactions taking place elsewhere on the molecule. The amino acid residues composing the binding region are being determined, as well as the position of these residues in the polypeptide chains which make up the protein.

Hybridomas in mice are being developed to provide homogeneous antibodies.

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430. MEMBRANE IMMUNOGLOBULINS AS RECEPTORS - STRUCTURE, FUNCTION AND CONTROL

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The membrane of a eukaryotic cell has, as integral components, glycoproteins which are receptors for a variety of regulatory molecules. Immunoglobulins are the specific membrane receptors for the antigens which stimulate proliferation and differentiation in B lymphocytes. Chemical characterization of these receptor immunoglobulins is of enormous interest, but the small amount of material and its relative insolubility in aqueous solutions have made this a difficult task. The recent development of microsequencing techniques based on detergent solubilization of the membrane molecules has made the detailed structural analysis of these molecules feasible. We intend to employ these techniques in the partial determination of the primary structure of membrane immunoglobulins from mouse B cell tumors. This information will be compared with similar information determined for secreted immunoglobulin and for membrane immunoglobulins from normal B cells. This detailed structural analysis will be a foundation from which to examine the functional interactions of the membrane immunoglobulins with other membrane molecules, using reversible cross-linking reagents. Changes in these interactions will be documented by comparing normal B cells, B cells stimulated to divide by mitogens, subpopulations of B cells at different developmental stages and B cell tumors. This system presents a unique opportunity for the study of chemical structure and function of an accessible, membrane-bound receptor which is intimately involved in the control of proliferation and differentiation in normal cells.

431. BIOCHEMICAL STUDIES ON MEMBRANE BOUND IMMUNOGLOBULIN

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The modes of attachment of cell surface proteins are being studied in mouse cells, trypanosomes and viruses. The methods used will include surface iodination using 125I as the labeling agent as well as conventional methods of proteolytic enzyme digestion and subsequent structural analysis of surface proteins. The aim of this work is to study the mechanisms responsible for the interaction of the membrane and its associated proteins.

432. STRUCTURE OF IMMUNOGLOBULINS

Poljak, R. J., Amzel, L. M., Amzel, V., Hirama, M., Johns Hopkins University, School of Medicine, Dept. of Biophysics, *725 N. Wolfe St., Baltimore, Maryland,* 21205, United States of America

Our studies are directed toward the determination of the three-dimensional structure of immunoglobulins and specific antibodies and the understanding of the structural basis of antibody function. The detailed three-dimensional structures of two Fab fragments, several Fab-ligand complexes and the pfc fragments of guinea pig IgG1 will be studied by X-ray diffraction techniques. A search for hapten-specific induced antibodies suitable for structural studies will be continued using cell hybrids.

BIBLIOGRAPHIC REFERENCES: Chiu, Y.Y., Lopez de Castro, J.A. and Poljak, R.J. Amino Acid Sequence of the VH Region of Human Myeloma Cryoimmunoglobulin IgG Hil. Biochemistry 18, 553-560 (1979). Amzel, L.M. and Poljak, R.J. Three-Dimensional Structure of Immunoglobulins. Ann. Rev. Biochem. 48, 961-997 (1979).

433. PHYSICAL CHEMISTRY OF ANTIBODY EFFECTOR FUNCTIONS

DeLisi, C., Segal, D., Dower, S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Mathematical Biology Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

Kinetic and thermodynamic studies of various antibody aggregates interacting with mobile Fc receptors on cell surfaces.

BIBLIOGRAPHIC REFERENCES: DeLisi, C.: Physical Chemical and Biological Implications of Receptor Clustering. In DeLisi, C., and Blumenthal, R. (Eds.): Physical Chemical Aspects of Cell Surface Events in Cellulr Regulation. New York, Elsevier North-Holland, 1979, pp. 261-285.

434. STUDIES ON SECRETORY IMMUNOGLOBULIN

Lamm, M. E., Rao, C. V., McWilliams, M., New York University, School of Medicine, Dept. of Pathology, *100 Washington Sq. E., New York, New York,* 10003, United States of America

We shall study the mechanism of homing of IgA precursor cells from gut-associated lymphoid tissue to secretory sites and attempt to demonstrate that such cells taken from orally immunized mice can transfer a specific IgA antibody response to the lactating mammary gland. We shall also study the influence and role of hormones in this phenomenon.

Other aspects of our work are concerned with how secretory component is covalently linked to IgA and whether a disulfide interchange enzyme could play a role in this process.

BIBLIOGRAPHIC REFERENCES: Lamm, M.E., Roux, M.E., Weisz-Carrington, P., McWilliams, M. and Phillips-Quagliata, J.M. 'Cellular Origin of the IgA in Milk' in Protides of the Biological Fluids-XXVth Colloquium, ed. H. Peeters, Pergamon Press, New York, 1978, p. 835. Weisz-Carrington, P., Roux, M.E., McWilliams, M., Phillips-Quagliata, J.M. and Lamm, M.E. Hormonal induction of the secretory immune system in the mammary gland. Proc. Nat. Acad. Sci. USA, 75:2928, 1978.

435. SECRETORY IGA SYSTEM - MOLECULAR-CELLULAR INTERACTIONS

Mestecky, J. F., University of Alabama in Birmingham, School of Dentistry, Dept. of Microbiology, *1919 7th Ave. S., Birmingham, Alabama,* 35233, United States of America

The objective of these studies is to determine the molecular and cellular interactions that lead to the selective expression of IgA in secretory glands and to the selective transport of IgA into external secretions. The structural basis of the binding of immunoglobulins to specific receptors on epithelial cells will be investigated and we shall attempt to identify and characterize the immunoglobulin binding receptor.

Properties of unstimulated and mitogen-stimulated colostral lymphocytes will be determined. With the use of immunofluorescence techniques, we shall determine the intracellular and membrane-associated immunoglobulin class. The presence of receptors for secretory component will be determined by immunofluorescence on the membrane and in cytoplasm of stimulated and unstimulated lymphocytes. The effect of co-cultivation of peripheral blood or colostral lymphocytes and normal or tumor epithelial cells on the expression of the immunoglobulin isotype will be studied. With the use of physicochemical techniques, conformational studies will be made of constituent components of secretory IgA molecules.

BIBLIOGRAPHIC REFERENCES: Mestecky, M., Preud'homme, J.-L., Crago, S.S., Mihaesco, E., Prchal, J. and Okos, A.J. Presence of J Chain in Human Lymphoid Cells. Clin. Exp. Immunol. 39:371, 1980. Jackson, S., Mestecky, J. and Bennett, J.C. Interspecies Idiotypic Cross-Reactivity of Anti-Albumin Antibodies. Fed. Proc. 39: No. 994, p. 456, 1980.

436. ORGANIZATION OF IDIOTYPICALLY SIMILAR IG VARIABLE REGION GENES

Gefter, M. L., Near, R. I., Massachusetts Inst. of Technology, School of Science, Dept. of Biology, *77 Massachusetts Ave., Cambridge, Massachusetts,* 02139, United States of America

Instead of myeloma models, we propose to study an idiotypic model of immunoglobulin gene expression. This model should more accurately reflect in vivo events and has known genetics. The model is based on the post-immunization production in all individuals in a mouse strain of antibody containing major idiotypic determinants which cross react. By making idiotypically positive hybridomas we can purify Ig-mRNA, synthesize cDNA, and create idiotypic clones.

Variable region cDNA from these clones will be used as hybridization probes in Southern blots to study: 1) if the major idiotype is represented by a germline gene, 2) if a family of such genes exist, and 3) how these genes are rearranged in going from germline to expressed (as examined in the genomic DNA of the hybridomas).

From the above data, various genomic pieces will be selected to be cloned into Charon 4A lambda phage vectors. These clones will be restriction mapped and selected pieces sequenced. From these sequences we hope to learn: 1) how families of v-genes vary in their sequences and how these variations relate to idiotypes, 2) what causes a certain member of a family to be expressed, and 3) how to define idiotypes at the DNA level.

437. IMMUNOGLOBULIN-RELATED MARKERS OF NORMAL AND NEOPLASTIC PRIMATE T LYMPHOCYTES

Marchalonis, J. J., Medical University of South Carolina, School of Medicine, Dept. of Biochemistry, *80 Barre St., Charleston, South Carolina,* 29401, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

438. CHARACTERIZATION OF ANTIBODY DIVERSITY (MICE)

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This is part of a broader project. A summary of this subproject is not available.

439. THE STRUCTURE AND CHEMICAL NATURE OF SPECIFIC ANTIBODY SITES (MICE)

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This is part of a broader project. A summary of this subproject is not available.

440. HORSE ANTIHUMAN THYMOCYTE GAMMAGLOBULIN

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This is part of a broader project. A summary of this subproject is not available.

B. ANTIBODY PRODUCTION AND REGULATION

441. STUDY OF IMMUNOGLOBULIN SYNTHESIS IN LEUKEMIA, LYMPHOMA AND IMMUNODEFICIENCY AND CORRELATION WITH NORMAL LYMPHOCYTE FUNCTION

Smith, J. L., HannamHarris, A. C., Southampton General Hospital, Dept. of Immunology, *Southampton, England, United Kingdom*

OBJECTIVES: (1) Subdivision of lymphocytic disease on the basis of the capacity of the neoplastic lymphocytes to synthesize immunoglobulin and in particular excess light chain in vitro. (2) Investigation of the relationship between the capacity of neoplastic lymphocytes to synthesize free light chain immunoglobulin and immunoglobulin synthesis by B cell populations from normal and immunodeficient individuals. (3) Investigation of cellular mechanisms of free light chain synthesis and secretion in immature neoplastic and normal B lymphocyte populations and early detection of this phenomenon in neoplasia as an indication of neoplastic disease.

APPROACH: Immunoprecipitation and polyacrylamide gel electrophoresis of 3H-leucine labelled supernatant and lysate immunoglobulin from normal and neoplastic cell cultures. Normal and neoplastic cell preparations from fetuses, normal spleen bloods and lymph nodes and from patients with immunodeficiency, chronic lymphocytic leukemia and non-Hodgkin's will be investigated. In some cases attempts will be made to modulate Ig synthesis patterns with T cell and/or mitogens.

442. SYNTHESIS OF ANTIBODY PEPTIDES THAT FIX COMPLEMENT

Erickson, B. W., Prystowsky, M. B., Munoz, H., Rockefeller University, Biochemistry Lab., *1230 York Ave., New York, New York*, 10021, United States of America

Complement fixation by immune complexes is the crucial link between the immunoglobulin recognition system and the complement effector system of human serum. Activation of the complement cascade by antigen-immunoglobulin G complexes involves a specific interaction between the CH2 domain of the antibody heavy chain and the globular head of the C1q complement component. This interaction induces activation of C1 through selective proteolysis of C1r and C1s. The subsequent steps in the complement cascade result in stimulation of inflammation, cell opsonization, and cell killing.

We have prepared synthetic peptides from the CH2 domain of IgG1 that both bind to C1 and inhibit the binding of IgG1 to C1. A hydrophobic pentapeptide containing tryptophan-277 and a basic decapeptide containing histidine-285 were each active and a 16-residue peptide containing both residues was even more active. Thus the binding site of IgG1 contains both a hydrophobic region and an ionic region. This project will explore the antibody-binding site of C1q by identifying the structural features of the CH2 site that facilitate binding. A series of radiolabeled peptides will be prepared by solid-phase peptide synthesis that define the roles of tryptophan-277 and histidine-285 in enhancing complement fixation. These synthetic peptides will themselves be antagonists of complement fixation. By dimerization in solution or by attachment to solid supports, these peptides will be converted into synthetic agonists that directly fix complement. In addition, analogues of these peptides will be prepared for covalent affinity labeling and identification of the humoral C1q receptor site for the CH2 domain of IgG.

BIBLIOGRAPHIC REFERENCES: Erickson, B.W. and Prystowsky, M.B. (1979) Continuous-flow solid-phase peptide synthesis using on HPLC apparatus, in Biological/Biomedical Applications of Liquid Chromatography II, C.L. Hawk, Ed., Marcel Dekker, New York NY., pp 293-305. Erickson, B.W., Lukas, T.J. and Drystowsky, M.B. (1980). Automated solid-phase peptide synthesis, in Polypeptide Hormones, R.F. Beers, Ed., Raven Press, New York, in press.

443. ARE CHANGES IN HEAVY CHAIN GENE ORGANIZATION INVOLVED IN CONTROL OF IMMUNOGLOBULIN BIOSYNTHESIS?

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During the course of a normal immune response to a given antigen, the predominant class of antibody synthesized changes from IgM to IgG. That is, the same variable region is expressed in conjunction with two different heavy chain constant regions at different times in the antibody-producing cell's history. I propose to ask how this occurs, and in particular, whether it involves a rearrangement of the DNA coding for the variable and constant region genes. I plan first to establish antibody-producing cell lines in which the same variable region is associated with each type of constant region. The heavy chain messenger RNA from each cell line will then be isolated, and a DNA copy of it cloned in a bacterial plasmid. Finally, the cloned message copies will be used as hybridization probes for the variable and constant region genes, to ask whether the physical arrangement of these genes differs in the IgM- and IgG-producing cell lines.

444. IDIOTYPE AND GENETIC CONTROL OF ANTIBODY BIOSYNTHESIS

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During the past two years we have developed methods for isolating large numbers of idiotype-specific suppressor T cells. We are now in the process of attempting to isolate a soluble suppressor factor from such cells. Preliminary work is promising but complete suppression has not yet been attained. We will therefore continue our studies, attempting to generate large numbers of specific suppressor T cells, and to isolate suppressive factors from the membrane or cytoplasm of such cells. In another line of investigation, we will provide specifically purified anti-Ar antibodies to Dr. J.D. Capra, who is carrying out amino acid sequence studies on their heavy chains. We will also provide him with heavy chains from hybridoma products which carry the cross-reactive idiotype and other closely related idiotypes which we have found to be present in immune sera. In a third line of investigation, we will extend our studies indicating that four strains, which synthesize light chains possessing a unique polypeptide marker, fail to provide the necessary light chains for the production of an idiotype associated with anti-phenylarsonate antibodies in A/J mice. The generality of this phenomenon will be determined by extending this work to other idiotypes characteristic of particular strains of mice. In each case the second strain used in the mating study will be one of the four strains which is characterized by the unusual light chain marker.

BIBLIOGRAPHIC REFERENCES: Laskin, J.A., Gray, A., Nisonoff, A., Klinman, N.R. and Gottlieb, P.O., 'Segregation at a Locus Determining an Immunoglobulin Genetic Marker for the Light Chain Variable Region Affects Inheritance of Expression of an Idiotype.' Proc. Natl. Acad. Sci. 74, 4600 (1977). Ju, S-T., and Nisonoff, A., 'Investigation of the B Cell Repertoire through the Study of Non-Cross-Reactive Idiotypes.' Immune System: Genetics and Regulation. E.E. Sercarz, L.E. Herzenberg and C.F. Fox, Editors, Academic Press, N.Y., p. 201 (1977).

445. IMMUNOREGULATION OF ANTIBODY SYNTHESIS

Dickler, H. B., Markman, M., Weissberger, H., Lubet, M. T., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Immunology Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Antibodies specific for the idiotype(s) of anti-(T,G)-A- -L antibodies of two different mouse strains (C57BL/10Sn and C3H.SW) have been obtained. The idiotype(s) of these two strains are distinct and the anti-(T,G)-A- -L antibodies of the majority of individuals of each strain express identical or cross-reactive idiotype(s). Since the ability of mice to respond to (T,G)-A- -L is under the control of H-2 linked Ir genes, these anti-idiotype antibodies are being used in conjunction with radioimmunoassay of the binding of (T,G)-A- -L to anti-(T,G)-A- -L antibodies as well as both in vitro and in vivo humoral response assays to evaluate the cellular and genetic control of idiotype expression as well as the role of idiotype-bearing molecules in regulation of the immune response. T lymphocytes express anti-(T,G)-A--L idiotype(s) identical to or cross-reactive with those found on anti-(T,G)-A- -L antibodies of the same strain. Expression of similar or identical idiotypes is found on both IgM and IgG antibodies, and expression of these idiotypes is controlled (at least in part) by genes linked to the heavy chain allotype locus.

BIBLIOGRAPHIC REFERENCES: Pincus, S. H., Singer, A., Hodes, R. J., and Dickler, H. B.: Anti-(T,G)-A- -L idiotypes: Initial studies of genetic control and cellular expression. In Pernis, B. and Vogel, H. (Eds.): Cells of Immunoglobulin Synthesis. New York, New York, Academic Press, 1979, pp. 371-380.

446. PRODUCTION OF TUMOR-SPECIFIC ANTIBODIES AGAINST MEMBRANE COMPONENTS OF HUMAN KIDNEY CARCINOMA

Birkmayer, G. D., Wieland, W., Schmidt, E., Ludwig Maximilians Universitat Munchen, Dept. of Cell Biology, *Goethestr. 33, D8000 Munich 2, Federal Republic of Germany*

OBJECTIVE: Preparation of a specific antibody against tumor-specific surface components from human kidney carcinoma by immunizing sheep which have been made tolerant to normal kidney antigens in their embryonic phase.

APPROACH: Embryonic sheep are immunized against a membrane extract from normal human kidneys in order to make them tolerant. About 6 weeks after birth the young sheep are injected with identical extracts from human kidney carcinoma from the same patient from which the normal tissue was taken. The serum is tested after each booster injection and the one with the highest titer will be selected for further purification of the antibody.

PROGRESS: Pilot studies demonstrated that sheep still produce antibodies against normal kidney components even though they are immunized in their embryonic life span. However, antibodies against the tumor components have a higher titre in the serum of the sheep preimmunized in their embryonic phase.

447. REGULATION OF IMMUNOGLOBULIN PRODUCTION AND MUTATIONS (HUMAN, MICE)

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This is part of a broader project. A summary of this subproject is not available.

448. THE RELATIONSHIP OF THE IMMUNE RESPONSE TO TUMOR DEVELOPMENT AND THE PREPARATION OF XENOANTISERA TO TUMOR SPECIFIC ANTIGENS

Levy, J. G., University of British Columbia, Dept. of Microbiology, *2075 Wesbrook Pl., Vancouver, British Columbia*, V6T 1W5, Canada

OBJECTIVES: To study immunoregulation of tumor immunity in a mouse tumor model (the P815 mastocytoma of DBA/2 mice). To purify tumor antigens from human tumors of various histological types.

APPROACH: An in vitro culture system for the generation of syngeneic cells cytotoxic for P815 cells has been developed and is being used for the study of antigen specific suppressor cells and their factors generated by tumor growth or exposure to tumor antigen.

Xenoantiserum, specific for apparently tumor unique components of human squamous cell carcinoma of the lung, has been prepared and used to monitor the purification of the tumor antigens.

PROGRESS: Suppressor cells and a soluble factor have been demonstrated in the thymuses and spleens of mice bearing P815 tumors which specifically suppress the in vitro generation of cells cytotoxic for P815. The cells can be killed by appropriate anti-Ia antiserum plus complement and the factor can be removed by adsorption with anti-Ia antiserum. Neither the cells nor the factor exhibits H-2 restriction when tested in radiation chimeras.

An antigen apparently unique to carcinoma of the lung has been isolated using the monitoring antiserum and standard biochemical procedures. A similar approach is being used to purify an antigen from P815 mastocytoma.

449. B CELLS DIRECTED AT SELF-ANTIGENS - ANTI-ERYTHROCYTE ANTIBODIES IN NATURAL AND INDUCED AUTOIMMUNITY

Knight, A., Knight, J., Mitchison, N. A., Winchester, G., Imperial Cancer Research Fund, Tumor Immunology Unit, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

The summary for this project is under entry no. 239.

450. PRODUCTION OF ANTIBODIES TO TUMOR ASSOCIATED ANTIGENS

Leung, K., Kardos, P., Rutgers, The State University of New Jersey, New Brunswick Campus, Waksman Inst. of Microbiology, *Waksman Hall, New Brunswick, New Jersey*, 08903, United States of America

Tumor associated antigens (TAA) are solubilized from plasma membranes of MST (Moloney sarcoma in BN rats), LM2 (Moloney sarcoma in Lewis rats), BC3 and BC5 (sarcomas in BN rats induced by methylcholanthrene). TAA are purified on controlled-pore-glass columns, yielding fractions devoid of viral antigens, fetal antigens and histocompatibility antigens. TAA will also be prepared from the cytosol of these cells by passing the cytosol through an immunoabsorbent column of anti-TAA antibodies. Rabbits and goats are immunized with membrane TAA and cytosol TAA. Cell hybrids are formed between mouse myeloma cells and rat immune spleen cells and are cloned for monoclonal antibodies to these TAA. These monoclonal antibodies will be used to form immunoabsorbent column and to further purify TAA. The relationship between membrane TAA and cytosol TAA will be determined by pulsing with radioactive amino acid followed by cold chase. The usefulness of these homogenous TAA, anti-TAA antibodies and biochemically modified TAA (conjugated to tubercle bacilli, DNP and dodecanoic acid) as vaccines and immunotherapy will be studied.

451. PREPARATION AND CHARACTERIZATION OF AN ANTISERUM AGAINST A HOST CELL 53K PHOSPHOPROTEIN WHICH COMPLEXES WITH SV40 LARGE-T

McCormick, F., Harlow, E., Imperial Cancer Research Fund, Translation Laboratory, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

We have purified a mouse 53K phosphoprotein by large-scale immunoprecipitation with an SV40 anti-tumor serum followed by SDS gel electrophoresis. Antibodies were raised in a rabbit against this purified, denatured protein. This anti-53K serum precipitates the 53K immunogen and SV40 large-T from lysates of SV40 transformed 3T3 cells. The serum has no detectable activity against large-T itself, and we believe it precipitates this protein through an association with the 53K mouse protein against which the serum was raised.

About one half of the large-T present in cell lysates can be immunoprecipitated by anti-53K serum, but all of the 53K protein is immunoprecipitated by anti-large-T serum. Thus all of the 53K appears to be bound to large-T.

The 53K-large-T complex and unbound large-T can be separated by density gradient centrifugation (see Report 71). The bound form co-sediments with 53K at 23S and is immunoprecipitated by anti-53K serum. The free form sediments at 5S and cannot be immunoprecipitated by anti-53K serum.

Indirect immunofluorescence studies have shown that the 53K-large-T complex is in the cell nucleus.

452. REGULATION OF THE RAT IGE SYNTHESIS

Bazin, H., Universite Catholique de Louvain, Faculty of Medicine, Experimental Immunology Unit, *4 Clos Chapelle-aux-Champs, 1200 Brussels, Belgium*

We plan to carry on our studies concerning the regulation of the IgE immune response. 1) Genetic control and regulation of the IgE reaginic antibody production. We are presently studying a possible linkage of the high IgE response with a known gene. This study aims at the discovery of criteria which would permit detection of humans with high risks of sensitization. We also plan to study the physiological mechanism(s) which stop(s) the IgE reaginic immune response. We use the radiation-induced enhancement model of the IgE immune response which we have developed. We hope to find a method with which to stop the reaginic immune responses in allergic patients. 2) Role of antibodies and in particular of IgE reaginic antibodies in the defense of organisms (rats) against bacterial and parasitic diseases. Using immunodeficient rats (athymic or neonatally mu-suppressed animals) we shall study the different cellular (macrophage, eosinophil, mast cell etc.) or humoral (antibodies of different isotypes) parameters of the defense mechanisms against parasites. 3) Rat myeloma tumor incidence. We shall pursue our studies of the genetic control of the rat myeloma tumor which is the only experimental model of such cancer.

BIBLIOGRAPHIC REFERENCES: Bennich H., Karlsson T., Bazin H. and Zeuthen J., In vitro culture of IgE secreting cells from two rat myelomas. Prot. Biol. Fluids, 1978, 25:559-562.

453. CELLULAR MECHANISMS REGULATING ANTIBODY PRODUCTION

Mullen, H. B., University of Missouri, Columbia Campus, School of Medicine, Dept. of Medicine/Microbiology, *M228*

Medical Sciences, Columbia, Missouri, 65201, United States of America

The objective of this research is to determine the cellular requirements for activation of antibody-producing (B) cells by thymus T dependent (TD) and T independent (TI) antigens by determining 1) the types of cells which are activated by TI antigens, in particular, whether TI antigens can activate memory B cells, suppressor T cells, or helper or amplifier T cells, and 2) whether T cells are required for the induction of IgG memory responses by TD antigens.

Antibody responses will be measured by the Jerne plaque assay and by passive hemagglutination. The effects of priming with TI antigens will be measured by transferring the cell type to be tested with appropriate cells from mice primed with TD antigens to irradiated recipient mice. The role of T cells in induction of IgG memory responses will be measured by transferring primed B cells from athymic nude mice to irradiated recipients together with syngeneic primed T cells.

454. ANTIBODY MEDIATED CELL-CELL INTERACTIONS

Phillips Quagliata, J. M., New York University, School of Medicine, Dept. of Pathology, *100 Washington Sq. E., New York, New York,* 10003, United States of America

The overall objective of the work proposed is to determine how IgA production is regulated in mice. We plan to determine whether the differentiation of bone marrow derived (B) cells into IgA producing plasma cells is regulated by special subsets of thymus-derived T cells, uniquely adapted for interaction with IgA precursor B cells, either by recognition of such cells or by preference for the same lymphoid organs, i.e., the gut-associated lymphoid tissue (GALT), or whether any T helper (Th) and T suppressor (Ts) cells of the appropriate carrier specificity will do. We shall examine the possibility that special T cells or non-lymphoid elements of GALT, specifically Peyer's patches, provide a differentiation signal, necessary for the differentiation of B cells, bearing mu and/or delta chains, to IgA production. We shall probe the question of whether there can be a secondary IgA response (i.e., whether there are IgA memory B cells) and whether there are T dependent and T independent antigens capable of stimulating IgA production. We shall attempt to determine how the selective migration of different populations of B and T lymphocytes into different lymphoid organs is controlled.

BIBLIOGRAPHIC REFERENCES: Weisz-Carrington, P., Roux, M.E., McWilliams, M., Phillips-Quagliata, J.M. and Lamm, M.E. Organ and isotype distribution of plasma cells producing specific antibody after oral immunization. Fed. Proc. 38:4511, 1979. Roux, M.E., McWilliams, M., Lamm, M.E. and Phillips-Quagliata, J.M. Site of maturation of IgA plasma cell precursors. Fed. Proc. 38:4512, 1979.

455. ANTITUMOR ANTIBODIES GENERATED IN VITRO

Levy, R., Finn, O. J., Dilley, J., Zipf, T., Stanford University, School of Medicine, Dept. of Medicine, *300 Pasteur Dr., Stanford, California,* 94305, United States of America

In this project we will be attempting to study cell surface antigens which are specific to tumors. For this purpose we have adapted a technique for the production of antibodies of restricted heterogeneity in vitro. The objectives here will be to define the antigenic relationships between different closely related murine lymphoid tumors, and to determine the degree of overlapping in the array of tumor specific antigens of these different tumors. In addition, we will be attempting to produce antibodies which recognize human tumor specific antigens, particularly in leukemia and lymphoma. These antibodies against human tumor antigens may find application in immunodiagnosis and immunotherapy.

456. CELLULAR INTERACTION REGULATING IMMUNOGLOBULIN PRODUCTION

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This is part of a broader project. A summary of this subproject is not available.

457. PRODUCTION OF SERUM ANTIBODY TO EPIDERMAL CELL SURFACE ANTIGENS IN MICE

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This is part of a broader project. A summary of this subproject is not available.

C. ROLE OF ANTIBODIES IN TUMOR IMMUNITY

458. TUMOR ASSOCIATED ANTIGENS AND IMMUNOGLOBULINS IN MAN

Dorval, G., Schulz, J., Wilkinson, R. D., Osterland, C. K., Shibata, H. R., Royal Victoria Hospital, Clinical Immunology Division, *687 Pine Ave. W., Montreal, Quebec,* H3A 1A1, Canada

OBJECTIVES: Isolation of melanoma neoantigens (TAA) from malignant effusions and from circulating immune complexes (IC). Identification of melanoma-associated antibodies (T-Ig) within sera and isolated IC from malignant melanoma patients and from individuals with vitiligo. Production of melanoma specific xenoantisera. Analysis of the dynamics of 'normal' versus 'malignant' surface antigenic components on melanoma cells. Immunologic monitoring of disease/treatment activity.

APPROACH: We will isolate melanoma-type specific neoantigens and proceed to molecular characterization of purified components with ascertained reactivity for T-Ig. Xenoantisera will be raised against 'promising' TAA samples and will be used for monitoring further the purification of TAA. Extensive adsorption of antisera will be done using normal and malignant tissue/cell lines. Specificity of the antisera will be assessed by inhibition of cytotoxicity of melanoma cells by different tumor extracts. Anti-TAA xenosera will then be used to study the turn-over of melanoma membrane components.

PROGRESS: We have now developed a micro-radioimmunometric assay capable of detecting 10 to the minus 12th power g of IC (modified from Yang et al., J. All. Clin. Immunol. 62:300, 1979). K-562 cells with 'blocked Fc-receptors' (using aggregated IgG) (HAGG) are first reacted with sera/fluids potentially containing T-Ig/TAA to remove any K-562 antibody activity. Sera and effusions are also adsorbed onto pooled, immunolized immunoglobulins and lymphocytes. They are then reacted in different ratios and incubated with 'fresh' K-562, which has a high density of Fc-receptors. Adsorption of IC is quantitated by radiolabeled S. aureus protein A. Using criss-cross specificity controls with non-malignant sera/effusions, T-Ig could be detected in 21/34 stage I melanoma patients, against a 300,000 d. fraction from a melanoma malignant effusion, but not against any other sera/effusions thus far tested.

459. HUMAN ANTIBODIES TO MELANOMA

Wright, P. W., Nowinski, R., Hanson, J., Berglund, C., Fred Hutchinson Cancer Research Center, Tumor Immunology Division, *1124 Columbia St., Seattle, Washington,* 98104, United States of America

In this application we propose to prepare a series of hybrid cell lines that produce monoclonal human antibodies against cell surface antigens of human melanoma cells. These hybrids will be prepared by the fusion of myeloma cells with the lymphocytes of melanoma patients who are undergoing an active immune response to their tumor cells. Culture fluids from the hybrid cells will be tested for serological activity against melanoma cells. For the initial screening procedure we have established a series of matched cell lines (melanoma cells, skin fibroblasts, and B cells) from eleven individual melanoma patients. Hybrid cells which produce antibodies that react selectively with the melanoma cells in vitro will be cloned and used as the source of homogeneous immunoglobulin. Subsequently, these monoclonal anti-melanoma antibodies will be examined further for specificity by immunofluorescene and immunoperoxidase tests with biopsied tissues that will be obtained from melanoma patients. In these biopsied samples we will be able to compare the reactions of the anti-melanoma antibodies with both tumorous tissues and the normal cell counterparts that reside in adjacent segments of the tissue.

460. ANTILEUKEMIA ANTIBODIES

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PURPOSE: To induce antibodies against human acute lymphoblastic, myeloblastic and myelomonoblastic leukemia cells and to use these antibodies as an aid in diagnosis and follow-up of patients with acute leukemia.

Diagnosis and follow-up of patients with acute leukemia have relied almost exclusively on the light microscopy of the blood and bone marrow. The current study hopes to add to these diagnostic armamentarium in a more specific fashion by use of the antibodies.

To date antisera have been raised in rabbits against a T-cell acute lymphoblastic leukemia and a null-cell acute lymphoblastic leukemia. However, their specificity has not yet been evaluated and may require further purification before their usefulness as diagnostic agents can be evaluated.

461. ANTI-II ANTIBODIES AND II ANTIGENS IN CARCINOMAS

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In a study of 170 breast cancer patients we detected significant increases in red blood cell cold agglutinin activities in Stage III, and the histological varieties of apocrine and mucinous carcinoma. Increased lymphocytotoxic activities were also found in breast cancer sera and this activity correlated significantly with red blood cell cold agglutinin levels. This suggests a possible role of these cold reactive antibodies in host defenses in cancer patients. Increased serum I antigen activities were found in Stage IV patients. A RIA method is being developed for precise quantitation of anti-I and I antigen in cancer sera. Under investigation are derivatization of the carbohydrate I antigen by tyramine for the purpose of labeling with 125I and the usefulness of a double antibody method. In the RIA we are planning to characterize immunochemically anti-Ii in cancer sera by oligosaccharide inhibition assays. The results may elucidate the biological role of cancer related anti-Ii and Ii.

462. LOCALIZATION OF ANTI-CEA ANTIGEN AND ANTI-CDP ANTIBODIES IN BREAST CANCER

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This is part of a broader project. A summary of this subproject is not available.

463. OVER-REPRESENTATION OF ANTIBODIES AGAINST IMMEDIATE ANTIGENS

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This is part of a broader project. A summary of this subproject is not available.

464. ANTIBODIES TO EB AND C-TYPE VIRUS IN GREENLAND ESKIMOS

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Eskimos have as high an incidence of nasopharyngeal carcinoma as the Chinese of Southeast Asia. The study will examine whether Eskimos, like the Chinese, often have titres of antibodies to Epstein-Barr virus and at the same time whether Eskimos have the same titre of antibodies to simian sarcoma/leukemia virus as the Danes.

465. HUMAN SPECIFIC ANTIBODY RESPONSES TO INFLUENZA VIRUS IN VITRO

Callard, R. E., Imperial Cancer Research Fund, Tumor Immunology Unit, *Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom*

A system for studying in vitro the humoral response of human peripheral blood lymphocytes (PBL) has been developed. Using influenza virus as antigen it has been shown that, following a six-day culture of PBL with virus, active secretion of newly synthesized antibody can be detected during an overnight incubation. Synthesized antibody is detected by a radioimmunoassay in which culture supernatants are incubated in microtiter plates coated with viral antigen and bound antibody is detected with a 125I-labelled antiglobulin. Using this system it has been shown that antibody response is T cell-dependent and that there is a genetic restriction of the T-non T interaction. This is in contrast to the findings in the non-specific pokeweed mitogen antibody synthesis system. In addition, differences in radiosensitivity of the specific and non-specific helpers have been demonstrated. We intend to use the system to examine cell-cell interactions in the human humoral response, to investigate further the restrictions on cell interactions and to extend our studies to immunosuppressed tumor patients. We will also study the response to other viruses, particularly herpes zoster, which presents a severe problem in cancer patients.

466. ORGAN SPECIFIC TUMOR LOCALIZING ANTIBODY

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Much human cancer originates in organs not vital to the life and well-being of the patient. Many of these malignant tumors retain to some extent the morphological and histological characteristics of the organs or tissues from which they are derived. We believe that such tumor cells are also likely to retain the antigenic characteristics of some of the normal cells of the tissues that underwent malignant change. If such an antigen is expressed on the surfaces of tumor cells, but not on cells vital to the life and well-being of the patient, this antigenic characteristic may provide a target for an attack on tumor by immunological mechanisms.

We are looking for such antigens in rats, by trying to prepare radioactive antibodies able, after i.v. injection, to localize with high specificity in tumors originating in mammary tissue, large intestine, pancreas, and thyroid tissue. Antibodies will be sought in xenogeneic antisera. Radioactive tumor localizing antibody will be used as an immunological probe to identify the corresponding antigens during tumor cell fractionation studies. Such isolated antigens will then be used as immunogens to produce sera containing a larger fraction of tumor localizing antibody.

467. THE LOCALIZATION OF METASTATIC ADENOCARCINOMA OF THE PROSTATE BY RADIOLABELLED PROSTATIC ACID PHOSPHATASE ANTIBODIES

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OBJECTIVE: The use of radiolabelled antibody against prostatic acid phosphatase to image prostatic metastases in lymph nodes and bone will be investigated.

APPROACH: To evaluate the feasibility of this concept a model system will be established. Employing prostatic acid phosphatase producing human tumor in athymic nude mice, the tissue distribution of xenogeneic radioantibody against prostatic acid phosphatase will be studied. If tissue specificity for human prostatic tumors in the model system can be shown, clinical trials in patients with adenocarcinoma of the prostate will be carried out. It is hoped that early lymphatic spread to the primary regional lymph nodes of the prostate will be shown. This would provide more accurate staging of the postatic cancer and allow more effective and rational treatment.

468. STUDIES ON DISTRIBUTION OF ANTIBODY IN ATHYMIC NUDE MICE CARRYING HUMAN TUMORS WITH DEFINED ANTIGENIC MARKERS

Bagshawe, K. D., Lewis, J. C., Charing Cross Hospital, Dept. of Medical Oncology, *Fulham Palace Rd., London, W6 8RF, England, United Kingdom*

OBJECTIVE: To determine the distribution in body tissues and body fluids (and, wherever possible, the concentration) of heterospecific antibody directed at well-characterized antigens in tumor cell membranes.

APPROACH: Human gastrointestinal tract cancers which are known to synthesize the carcinoembryonic antigen are being grown in athymic nude mice. Highly purified gammaglobulin from animals immunized with CEA will be injected intravenously into the tumor carrying nudes. Body fluids, including extracellular fluid from normal sites and tumor sites, are being collected at various stages after intravenous administration to determine the concentration of antibody at these sites. Antibody concentration will also be determined by tissue excision and appropriate counting methods. Attempts will be made to determine the nature of antibody binding to tumor cells, in particular, whether this is specific or non-specific in nature.

Similar antibody coupled to potential cytotoxic agents will be similarly studied.

469. LOCALIZATION OF LABELED ANTIBODIES IN TUMORS (HUMAN, MICE)

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This is part of a broader project. A summary of this subproject is not available.

470. ANTIBODY-LINKED CYTOTOXIC AGENTS IN THE TREATMENT OF CANCER

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Our objective is to use anti-tumor antibodies as carriers of cytotoxic agents leading to preferential localization in tumors and selective destruction of malignant cells. This project stems from our demonstration that chlorambucil-linked anti-tumor globulins caused greater tumor inhibition in vivo and in vitro than antibody or drug alone.

The aims of this proposal are: (1) Develop a wider range of methods for binding different anti-cancer drugs to immunoglobulins; (2) improve the specificity and purity of carrier antibodies; (3) enhance localization of drug-antibody conjugates at target sites; and (4) assess tumor inhibition by conjugates in clinically pertinent models, e.g., animals with micrometastases and human tumors in nude mice.

Tumors vary in sensitivity and may acquire resistance to a given agent. Therefore, it is important to learn which agents will be active when administered as antibody conjugates and which chemical linkage will give greatest tumor inhibition. This should identify optimal drug-linkage combinations and develop guiding principles for this approach to cancer therapy.

Agents under study are adriamycin, which binds to DNA, alkylating agents, e.g., trenimon and chlorambucil, and methotrexate, an anti-metabolite. Coupling methods include both direct covalent and non-covalent linkages between drug and antibody and linkage via spacers, e.g., polylysine and dextran. Having demonstrated that progressive localization of antibodies against tumor-associated transplantation antigens (TAAs) depends upon the content of specific antibody, we are continuing efforts to obtain preparations enriched in anti-tumor globulins. Methods include: (1) Initial immunization with TAA-enriched fractions, (2) affinity chromatography of antisera, and (3) production of monoclonal antibodies against TAAs.

471. ANTIBODIES AS POTENTIAL TARGETING AGENTS IN PROSTATIC CANCER

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Present attempts to increase cytotoxic agents' selectivity with respect to tumor tissue include binding of the cytotoxin to antibodies which are specific for the tumor. The purpose of this proposed investigation is to examine the usefulness of antibodies directed against normal tissue-specific antigens of non-vital tissue, i.e., prostate, either singly or in conjugation with antitumor antibodies in destroying prostatic carcinoma cells. This investigation will examine the distribution and cytotoxicity of antibodies against prostate-specific acid phosphatase, prostate steroid binding protein, and other prostate tissue-specific antigens. The amount of specific uptake of these antibodies will be determined in vivo by normal prostatic tissue in the rat and dog and in prostatic tumors in the rat. The amount of tissue-specific antigen uptake will also be determined following injection of antisera directed against cell surface antigens. In addition to standard methods of antibody production and in order to generate the amount of antibody required for chemotherapeutic targeting, we will use myeloma fusion with cells extracted from spleens of antigen sensitized mice. This may provide us with monoclonal antibody.

In in vitro experiments with rodent and human prostatic tumors, we will examine complement and cell mediated tumor cell lysis in the presence of antisera by the use of chromium release procedures.

Initial tumor targeting will be performed with non-covalent adsorbed chlorambucil. The effects of this adsorbed material will be examined both in vivo and in vitro.

472. LOCALIZATION AND KILLING OF LYMPHOMA CELLS BY ANTIBODY

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OBJECTIVE: To prepare derivatives of tumor-specific antibody which can a) localize lymphoma masses by gamma imaging, and b) be employed in therapy of lymphocytic neoplasms.

APPROACH: We have developed a method for routine preparation of xenogeneic antibodies to idiotypic determinants on the surface Ig of neoplastic B lymphocytes. The antibody is effectively tumor-specific.

Gamma imaging of lymphoma masses by radiohalogenated antibody will be carried out in the guinea pig L2C leukemia and later applied to human lymphoma. Variables will include different radiohalogens, the use of Fab in place of whole antibody, and the attachment of halogen via cysteine in place of the conventional substitution on tyrosine.

Immunotherapeutic uses of antibody derivatives are also being assessed initially in the guinea pig, and will be assisted by data from gamma imaging and from studies of antibody cytotoxicity in vitro. Particular attention will be paid to the possibility of using radiohalogenated Fab fragments of antibody to penetrate lymphoma masses.

473. RADIOLABELED ANTIBODY TUMOR LOCALIZATION

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The goal is to prepare radiolabeled tumor-specific antibodies and to determine if these antibodies localize preferentially in tumors and metastatic tissue in animals. Tumor-specific antibody localization will be explored in BALB/c mice bearing syngeneic SV40 DNA virus-transformed tumors (SVT2). Irradiated cells in Freund's adjuvant will be injected at weekly intervals and sera collected one week after the last immunization, or viable SVT2 cells will be inoculated in the footpad and sera collected from animals with progressively growing tumors at six weeks. Groups of immunized and non-immunized mice will be inoculated with SVT2 and several other tumors of BALB/c mice to test the strength and specificity of the immune reaction. Sera from mice immunized with SVT2 will be tested for tumor-specific antibody activity using the isotopic antiglobulin technique developed by Harder and McKhann. The gamma globulin will be separated from the serum by ammonium sulfate precipitation and this material labeled with 125I. Normal gamma globulin will be labeled with 131I and used as a paired label control. Purification of the labeled antibodies will be by absorption and elution from the syngeneic SVT2 tumor cells; the cell bound antibody will be eluted in glycine acid buffer and dialyzed. Labeled normal gamma globulin will be freed of antibody aggregates by passage through normal BALB/c mice or Sephadex G-200 passage. In vitro binding specificity will be tested by absorption to syngeneic tumor cells and normal cells and by radioimmunoelectrophoresis using 131I-labeled rabbit or goat IgG antimouse gamma globulin fractions. In vivo localization of the labeled materials will be evaluated by tissue sampling and using a well-counter for determining radiolabel content. Labeled tumor-specific antibodies will also be produced for spontaneous tumors in cats with lymphoma. The animals will be immunized after tumor excision with weekly inoculations of inactivated tumor cells. Sera will be tested for tumor-specific antibody activity, then purified and radioiodinated, and assayed for in vitro binding specificity. The in vivo distribution of paired labeled antibody and normal gamma globulin will be studied by external scintigraphic imaging and emission CT imaging or by tissue sampling in cats with advanced tumors of the same and different histological types and the extent of the tumors will be studied at necropsy following death of the animals.

474. IN VIVO LOCALIZATION OF LABELLED TUMOR-SPECIFIC ANTIBODY FOR DIAGNOSTIC SCANNING AND FOR THERAPY

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OBJECTIVE: To study the interaction of anti-tumor antibody with tumor cells and endeavor to obtain a degree of localization in animal tumors suitable for the purpose of carrying scanning agents or the tumor.

APPROACH: Antibody to L5178Y mouse lymphoma has been prepared in a goat with partially purified tumor antigen. This was obtain by precipitating from 3M KCL extract of tumor cells those antigens which correspond to the antibodies in an anti-normal spleen cell serum. IgG was prepared by affinity chromatography. The time course of injected 125 I-labelled antibody in the tumor, blood and organs of mice was observed. The inactivation of antibody by cells has been studied in vitro. Antibodies released by tumor cells under normal culture conditions have, to a large extent, lost the ability to rebind to tumor cells. Degradation of antibody is evident from fractionations on Sephadex G-200. It is intended to produce large quantities of tumor-specific high affinity antibody by fusion of antibody producing spleen cells from an immunized mouse with a myeloma cell line. 211 AT amd 125 I will be used as agents to be coupled to antibody.

PROGRESS: A number of different immunosorbents have been tested for the purification of antibody to L5178Y mouse lymphoma. For an IgG preparation with an antibody content of 45% the tumor to blood concentration ratio was about 1 compared to an expected ratio of about 10. A rapid removal of antibody (compared to normal IgG from the tumor-blood system has been observed. This indicates that factors besides the purity of antibody limits the localization of antibodies in tumors. Antibody released from tumor cells in vitro has been shown (on filtration to Sephadex G-200) to contain antigen-antibody complexes and enzymatic degradation of IgG. Experiments in progress are aimed at the production of hybrid cells of antibody producing B cells from an immunized mouse and/or myeloma cell line.

475. RADIOLABELING OF TUMOR ANTIBODIES

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The objective of this proposal is to develop a technique for labeling antibodies with metallic radionuclides and to determine the relationship between accelerated blood clearance (caused by increased chelating groups per antibody) and the retention of immunology activity. In preliminary studies we found that the use of monomer albumin, the use of a conjugation method that assures a single type of linkage, and purification of the radiolabeled substrate did not prevent accelerated plasma clearance. The major factor controlling plasma clearance appeared to be the level of conjugation per antibody molecule. Thus we propose to study the effect of various reaction conditions including the conjugation method to determine if our approach to produce better tumor imaging agents by balancing altered substrate specificity caused by modification against accelerated plasma clearance is valid. If successful, this methodology will allow not only increased specificity in tumor detection because of the retention of immunologic activity but also a method to permit the use of earlier imaging times and consequently better radionuclides than presently employed with the agent of choice Ga-67 citrate.

476. LABELED ANTIBODIES AND TUMOR LOCALIZATION (HAMSTERS, MONKEYS)

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This is part of a broader project. A summary of this subproject is not available.

477. DETECTION OF EARLY ANTIBODIES TO MAMMARY CARCINOMA

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The early antibody response to tumor-associated antigens is being studied in an experimental animal tumor system. Mammary tumor incidence in females neonatally infected with the mammary tumor virus (MTV) is very high in some strains of mice and low in others. Thus, both retrospective studies for the characterization of antibody responses and prospective studies for testing antibody patterns predictive of eventual tumor development are feasible.

Serum samples from females of a strain with high tumor incidence (neonatally infected with MTV) are collected and frozen at regular intervals over the life span of the animals. As each female develops a mammary tumor which can be used to provide target tumor cells for in vitro testing, the serum samples are tested for the presence of antibodies directed against the various tumor-specific and virus-associated tumor antigens. A number of detection procedures are used, such as tests for blocking factors, cytotoxic antibodies, and virus-precipitating antibodies.

Since females bearing autochthonous rather than transplanted tumors are being used, the immune response in the natural course of a disease is being studied. These tumors are induced by a virus, and we suspect that part of the antibody response may result from a host-virus interaction which is not predictive of eventual tumor development; use of the model system will permit a qualitative as well as quantitative analysis of a complex response which may be directly applicable to human tumor systems.

478. EARLY DETECTION OF ANTIBODIES TO SYNGENEIC MOUSE TUMORS

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OBJECTIVE: Study alterations in the serological reactivity pattern in mice at high risk of developing carcinogen-induced or endogeneous virus-induced malignancies as well as in reactivity of such mice to antigens associated with the relevant tumors. Model systems are to be: spontaneous tumors in C3Hf mice; mammary tumors induced by DMBA in BALB/c mice; and lung adenomas appearing in urethane-treated BALB/c mice.

479. ANTIBODY RESPONSES OF TUMOR BEARERS TO THEIR TUMOR ASSOCIATED ANTIGENS

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Tumor-associated antigens (TAA) on the surface of tumor cells growing in vivo may induce cellular and antibody-mediated immunological reactions in the tumor-bearing host. These reactions may inhibit or enhance tumor growth at local sites or be involved in facilitating or preventing spread, implantation and survival of tumor metastasis. The major objective of this proposal is to analyze the antibody responses of tumor-bearing mice to TAA on their own growing tumors by 'capturing' and perptuating part of the host's anti-TAA antibody repertoire in the form of hybridomas secreting monospecific anti-TAA antibodies. These monospecific antibodies will be used to identify and study TAA on a series of recently derived immunogenetically well characterized chemically induced murine tumors. Known TAA on these tumor lines include tumor specific transplantation antigens (TSTA), histocompatibility antigens (H-2 and non-H-2) and murine leukemia virus-associated antigens. The study includes the following additional objectives: 1. To compare the anti-TAA antibody responses of the tumor bearing host to syngeneic tumors classified as 'immunogenic' or 'non-immunogenic' based on TSTA strength. 2. To determine, using specific monoclonal antibodies, the antigenic relationship of tumor cells found in metastatic foci to those cells present in the original parent tumor. 3. To examine the antibody response of mice to their primary methylcholanthrene-induced tumors before the tumors are transplanted for the first time. 4. To assess in a preliminary way several monoclonal anti-TAA antibodies as mediators of anti-tumor immune reactions, particularly in antibody dependent cellular cytotoxicity reactions.

480. EFFECTS OF LYMPHOCYTE-DEPENDENT ANTIBODIES ON TUMOR INDUCTION AND METASTASIS

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It is well established that tumor cells can be killed in vitro by specific lymphocyte-dependent antibodies (LDA) plus nonimmune effector cells, i.e., by antibody-dependent cell mediated cytotoxicity (ADCC), as we reported originally in 1972. Whether LDA can be utilized for control of tumor growth in vivo, however, is not yet clear. We propose to look for in vivo effects of LDA in two experimental models in which there is minimal tumor load; that is, in cases where the host is unlikely to have high levels of circulating immune complexes which could inhibit ADCC. The two models chosen for study in BALB/c mice are (1) primary sarcomas induced by 3-methylcholanthrene and (2) pulmonary metastases produced by intravenously injected tumor cells. In attempts to inhibit growth of metastases, mice will be treated with LDA having specificity for the tumor-specific transplantation antigens (TSTA) of the metastatic cells or for their common, cross-reacting oncofetal antigens (OFA). In the prevention protocol, anti-OFA sera will be administered during the latent period. Sera will be obtained from syngeneic tumor-immune or multiparous mice and from rabbits immunized with 10-14 day old BALB/c fetuses. Antibodies will also be obtained from cultured immune syngeneic spleen cells using technics described previously. Antibodies will be pretested for specificity in a binding assay utilizing iodinated protein A of Staphylococcus aureus and for LDA activity in a 51Cr release assay which uses tumor or fetal antigen-coated chicken red blood cells as targets. Recipients of antibodies will be monitored for the levels of circulating antibody using the IPA assay.

481. ANTIBODY AND CELL-MEDIATED SUPPRESSION IN SYNGENEIC TUMOR AND ALLOGENEIC MODELS

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Two major mechanisms, thought responsible for depression of specific anti-tumor immunity, are suppression by antigen-antibody complexes and downward regulation of immune responses by suppressor cells. Powerful immunosuppression induced by small quantities of antibody is Fc-dependent, not due to antigen-masking, often involves the induction of suppressor cells, and operates through the elimination of responding cells. We will employ the DBA2-anti-DBA2 mastocytoma system to investigate the interrelationships between inhibition by antibody and suppressor cell function. In order to provide a defined immune system which can be manipulated with respect to the amount of antigen present on stimulator cells and the class, specificity and affinity of antibody, we will employ TNP-modification and anti-TNP antibody to suppress a syngeneic anti-tumor immune response. TNP-modification and anti-TNP antibody have been shown to be effective in suppressing allogeneic immune responses through a mechanism not dependent upon antigen-masking and requiring an intact Fc portion of inhibitory antibody. If anti-TNP antibody inhibits TNP-modified tumor cells from eliciting a syngeneic anti-tumor immune response, we will determine whether or not suppressor cells appear and if removal of cell populations with abilities to generate suppressor cells modifies the ability of anti-TNP antibody to inhibit the syngeneic anti-tumor response stimulated against TNP-modified tumors. The role which Fc-receptors play in antibody-induced triggering of suppressor cells will be studied by removing cells with Fc-receptors. Lastly, the in vivo effect of cytotoxic and suppressor cells will be analyzed, using tumor curative procedures. Regulatory mechanisms which we define in the in vitro systems will be looked for in studies on tumor rejection in vivo.

482. THE ROLE OF ANTI-TUMOR ANTIBODIES AND TUMOR CELL PRODUCTS IN TUMOR IMMUNITY

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OBJECTIVE: Studies undertaken in our laboratory and elsewhere have shown that appreciable amounts of immunoglobulin may be associated with solid tumors in vivo. The aim of this project is to determine the characteristics of this association and to ascertain its possible relevance to tumor growth.

APPROACH: There are 2 aspects to this project. The first involves the quantitation of host immunoglobulins in tumor extracts and eluates and the second will determine the specificity of this association.

Cell suspensions will be prepared from freshly excised experimental (and possibly other) tumors using procedures which do not strip immunoglobulin from the cell surface. These suspensions will then be extracted with NP40 or eluted with low pH glycine HCl buffers. The amounts of the various immunoglobulin isotypes in the extracts and eluates will be determined using sensitive double antibody radioimmunoassay techniques developed in our laboratories (James et al. Cancer Immunology and Immunotherapy, 5, 141-143 (1978); James et al. British Journal of Cancer (In Press, 1979)). The interaction of host immunoglobulins with specific antigens on tumor cell surfaces will be investigated in a number of ways. In the first instance the association of immunoglobulin with freshly excised tumor will be examined by performing PAGE/SDS gel analyses on NP40 extracts of isotopically labelled tumors before and after separation on anti-Ig columns. Furthermore the ability of acid eluted Ig to combine with antigens on the surface of cultured tumor cells will be investigated using a combination of PAGE/SDS gel and affinity chromatography techniques. Similar studies will also be performed with antitumor antibodies produced by injection of X-irradiated tumor cells into syngeneic and allogeneic recipients.

483. CYTOTOXIC ANTITUMOR ANTIBODIES

Philpott, G. W., Parker, C. W., Kulczycki, A., Margraf, H. W., Washington University, School of Medicine, Dept. of Surgery, *660 S. Euclid Ave., St. Louis, Missouri*, 63110, United States of America

As a means of obtaining more efficient immunotherapy of tumors, we are investigating possible methods for increasing the cytotoxicity of antibodies. Antitumor antibodies have been conjugated to various toxic agents such as radioactive iodine, diptheria toxin, nitrogen mustards and enzymes with subsequent conversion of protoxins to toxins. Selective cytotoxicity has been demonstrated in a number of antitumor antibody-tumor cell models in vitro. These promising systems are being tested in vivo with immunoglobulin-enzyme conjugates in several tumor models in hamsters and mice. Extensive clearance and localization studies with antibodies, antibody fragments and immunoglobulin E are also being conducted to find ways of improving selective tumor localization. Purification of antibody-enzyme conjugates is also being attempted with affinity chromatography techniques.

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484. THE ROLE OF ANTI-IDIOTYPE ANTIBODY IN TUMOR GROWTH

Baird, L. G., Brandeis University, Rosenstiel Basic Medical Sciences Research Center, Dept. of Biology, *415 South St., Waltham, Massachusetts*, 02154, United States of America

The overal goal of this investigation is to determine whether all mice respond to a syngeneic mammary adenocarcinoma with the production of antibody bearing a cross-reactive idiotype. If antibody with a cross-reactive idiotype is shown, we will attempt to correlate the production of the cross-reactive idiotype and auto-anti-idiotype antibody with the modulation of tumor growth in animals bearing progressing or regressing tumors. This would suggest a possible normal regulatory role for idiotype and anti-idiotype in the modulation of tumor growth.

Initially, specific anti-tumor antibody will be raised in tumor bearing mice and purified by elution from tumor cells. This specifically purified antibody will be used to produce heterologous anti-idiotype antibody in rabbits. The anti-idiotype antibody will be used in a radioimmunoassay to determine whether tumor bearing mice produce antibody bearing a cross-reactive idiotype. Subsequently, tumor bearing mice will be treated with exogenous anti-idiotype antibody in an attempt to modulate the growth of tumors in these animals. Later experiments will consist of correlating the effect of anti-idiotype antibody on tumor growth in vivo with the effect on in vitro macrophage mediated tumor cytotoxicity and delayed type hypersensitivity.

485. STIMULATION OF TUMOR CELL GROWTH BY ANTIBODY

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The research project is concerned with exploring the fundamental molecular events taking place in the enhancement of tumor cell growth by antibody.

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486. ANTITUMOR ANTIBODIES GENERATED IN VITRO

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The summary for this project is under entry no. 455.

D. OTHER STUDIES ON ANTIBODIES

487. ANTIBODY STUDIES OF PROTEIN KINASE LOCATION IN RAT AND HUMAN MAMMARY CARCINOMA

Kraft, A., Cho Chung, Y. S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Pathophysiology, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

Antibodies to the regulatory subunit of protein kinase will be used to study the location of this protein before and after normally induced regression of mammary carcinoma. A procedure has been developed to isolate this protein in pure form in order to make antibodies.

488. NEW ANTIGENS AND ANTIBODIES AND THEIR RELATIONSHIP TO CANCER

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This is part of a broader project. A summary of this subproject is not available.

489. EFFECTS OF RABBIT ANTIBODY TO HUMAN TRANSFER FACTOR

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This is part of a broader project. A summary of this subproject is not available.

490. ROLE OF CELL-BOUND ANTIBODY IN IMMUNOREGULATION

Setcavage, T. M., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York,* 10021, United States of America

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493. SURFACE DISPOSITION OF CELL BOUND IGE WITH TIME

Triche, T. J., Isersky, C., Mims, S. J., Rivera, J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Ultrastructural Pathology Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

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494. ANTIBODY AFFINITY IN IMMUNE RESPONSE AND TOLERANCE

Siskind, G. W., Kim, Y. T., Dhabhar, P. J., Cornell University, Medical Center, Cornell Medical College, Dept. of Medicine, *1300 York Ave., New York, New York,* 10021, United States of America

Recently we have shown that thymus cells are required for, or facilitate, the differentiation of the B-cell population to be capable of producing a heterogeneous, high affinity antibody response to T-dependent antigens. This role of the thymus in B-cell differentiation will be investigated to determine: (a) the cell type involved; (b) whether the effect is mediated by a soluble factor; (c) whether the thymus has a similar role in regulating the differentiation of the B-cell population to produce a heterogeneous, high affinity antibody response to T-independent antigens; (d) whether thymus cells are involved in regulating the maturation of the B-cell population to be relatively resistant to tolerance induction; and (e) whether thymus cells are involved in regulating the maturation of the B-cell population to be capable of resynthesizing surface immunoglobulin following capping and shedding. Finally we will study the T-cell regulation of the changes in antibody affinity and heterogeneity which take place during the immune response.

495. ANTIBODIES TO TRANSITION STATES AS ENZYMES

Gilbert, H. F., Baylor College of Medicine, School of Medicine, Dept. of Biochemistry, *1200 Moursund Ave., Houston, Texas,* 77025, United States of America

The induction of a specific enzyme activity in the serum of mammals could be of benefit in the treatment of a large number of disorders. The specificity of antibody-hapten combining sites will be used to induce 'enzyme'-like activity toward a specific target chemical reaction in an antibody. A transition state analog-protein conjugate will be utilized to induce formation of antibodies which possess a combining site complementary to the transition state for the target reaction. The isolated antibodies should function as enzymes, catalyzing the target reaction by approximating or orienting the substrate(s) correctly at the active site. The target reactions include the bimolecular reaction of phenoxides with sulfonium salts and the Claisen rearrangement of allyl enol ethers. Neither reaction is subject to covalent or acid-base catalysis. The transition state analog hapten for the methyl transfer reaction will contain a carbon-carbon triple bond which will mimic the linear transition state of an SN2 reaction. For the Claisen rearrangement the transition state analog will be a substituted adamantane in which the carbon atoms are fixed into a cyclohexane chair conformation. Appropriate controls will be performed to insure that any observed 'enzyme' activity is due to reaction at the active site of the induced antibody.

496. USE OF SPECIFIC ANTIBODIES TO IDENTIFY RNA-DNA HYBRID REGIONS

Stroller, D., Tufts University, Boston Campus, School of Medicine, Dept. of Medicine, *136 Harrison Ave., Boston, Massachusetts,* 02111, United States of America

This is part of a broader project. A summary of this subproject is not available.

497. PROLONGATION OF GRAFT SURVIVAL WITH BLOCKING ANTIBODIES (DOGS)

Fortner, J. G., Sloan Kettering Inst. for Cancer Research, *410 E. 68th St., New York, New York,* 10021, United States of America

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498. SEARCH FOR ANTIGENS AND ANTIBODIES

Thiel, H., Duke University, School of Medicine, Dept. of Surgery, *Box 3711, Durham, North Carolina,* 27710, United States of America

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499. ROLE OF ANTI-IDIOTYPIC ANTIBODIES

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whether tumor bearing mice produce antibody bearing a cross-reactive idiotype. Subsequently, tumor bearing mice will be treated with exogenous anti-idiotype antibody in an attempt to modulate the growth of tumors in these animals. Later experiments will consist of correlating the effect of anti-idiotype antibody on tumor growth in vivo with the effect on in vitro macrophage mediated tumor cytotoxicity and delayed type hypersensitivity.

485. STIMULATION OF TUMOR CELL GROWTH BY ANTIBODY

Shearer, W. T., Baylor College of Medicine, School of Medicine, Dept. of Pediatrics, *1200 Moursund Ave., Houston, Texas*, 77025, United States of America

The research project is concerned with exploring the fundamental molecular events taking place in the enhancement of tumor cell growth by antibody.

BIBLIOGRAPHIC REFERENCES: Hartman, B.K., Agrawal, H.C., Kalmbach, S., and Shearer, W.T.: A comparative study of the immunohistochemical localization of basic protein to myelin and oligodendrocytes in rat and chicken brain. J. Comp. Neurol. 188:273-290, 1979. Tanner, D.D., Buckley, P.J., Hong, R., and Shearer, W.T.: Fatal cytomegalovirus bronchiolitis in a patient with Nezelof's syndrome. Pediatrics 65:98-102, 1980.

486. ANTITUMOR ANTIBODIES GENERATED IN VITRO

Levy, R., Finn, O. J., Dilley, J., Zipf, T., Stanford University, School of Medicine, Dept. of Medicine, *300 Pasteur Dr., Stanford, California*, 94305, United States of America

The summary for this project is under entry no. 455.

D. OTHER STUDIES ON ANTIBODIES

487. ANTIBODY STUDIES OF PROTEIN KINASE LOCATION IN RAT AND HUMAN MAMMARY CARCINOMA

Kraft, A., Cho Chung, Y. S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Pathophysiology, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Antibodies to the regulatory subunit of protein kinase will be used to study the location of this protein before and after normally induced regression of mammary carcinoma. A procedure has been developed to isolate this protein in pure form in order to make antibodies.

488. NEW ANTIGENS AND ANTIBODIES AND THEIR RELATIONSHIP TO CANCER

Hann, W. W., Inst. for Cancer Research, *7701 Burholme Ave., Philadelphia, Pennsylvania*, 19111, United States of America

This is part of a broader project. A summary of this subproject is not available.

489. EFFECTS OF RABBIT ANTIBODY TO HUMAN TRANSFER FACTOR

Finkelstein, M., New York University, School of Medicine, Dept. of Medicine, *100 Washington Sq. E., New York, New York*, 10003, United States of America

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